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CLINICAL AND EPIDEMIOLOGICAL STUDIES ON RICKETTSIAL  
INFECTIONS IN ETHIOPIA

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ANNUAL REPORT

1 OCTOBER 1977

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BY

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D. PROGRESS REPORT AND CURRENT STATUS

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\*This report is an extract from our annual contract-renewal application; hence the pagination. It is a complete entity, since it was planned for use as a formal annual report.

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D. PROGRESS REPORT AND CURRENT STATUS

1. BACKGROUND

a. The main endeavor of this project continued to be research on the ecology of murine typhus in Ethiopia, with corollary studies on the ecology of tick-borne rickettsiosis. The approach of sending a small team to undertake intensive field-work on these subjects, followed by detailed and exhaustive investigations in our Baltimore laboratory on the identification and analysis of the specimens and data collected, has proven to be a sound one. Once again the studies have proven to be highly fruitful, as indicated by the results reported herein.

b. The field team consisted of C.L. Wisseman, Jr., M.D., Director and Physician, R. Traub, Ph.D., as interim Field Director, and Entomologist, A. Farhang-Azad, Ph.D., as Microbiologist and D.A. Schlitter, Ph.D., as Mammalogist. The first 3 are from our Department, and Dr. Schlitter, of the staff of the Carnegie Museum, Pittsburgh, PA, is collaborating with us on this project. The team operated in Ethiopia during the period February - early March 1977, and thanks again to the splendid cooperation and support wholeheartedly rendered by NAMRU-5 in every respect, the investigators were able to spend virtually the entire period on constructive operations in the field. Hundreds (or thousands) of specimens of frozen material (sera, tissues and ectoparasites); dried samples of blood on filter paper; smears of microdissections of fleas, lice, ticks and mites; mammals and miscellaneous ectoparasites were collected, preserved or prepared, and observations and data amassed, all for critical follow-up study in the U.S.A.

c. Studies were also continued on earlier and current phases of the program in the light of new data and ideas. In the base laboratory at Baltimore, generally at no expense to the Contract, a series of experiments were undertaken on experimental infection with R. mooseri and other aspects pertinent to our understanding of murine typhus infection, and considerable effort was expended in preparing antigens, tissue cultures, conjugates, reagents and other expensive and time-consuming materials so vital to the project. The large and critical review of the ecology of murine typhus, containing new and original concepts, has been accepted for publication by the Tropical Diseases Bulletin, and manuscripts on the so-called extra-human cycle of epidemic typhus have been revised on the basis of subsequent material.

2. THE ECOLOGY OF MURINE TYPHUS

a. It is now firmly established that 1) with the new methodology it is possible to rapidly and accurately obtain data on rickettsial infections in mammals, fleas, lice, ticks and mites, and 2) the findings to date have continued to provide a firm basis for elucidation of the many unresolved questions about the ecology of murine typhus. All the evidence indicates that not only is this infection naturally restricted in Ethiopia to commensal rodents and their ectoparasites, but it is limited to only certain species thereof, i.e. Rattus rats and at least their fleas and lice, and perhaps Mus musculus, and does not occur in the wealth of native murines and ectoparasites infesting huts and buildings in many parts of the country unless these introduced murines are also present. If infection co-exists in a native murine and Rattus, it is apparently an unusual event in the autochthonous species, even in the same domicile. Moreover, since Rattus and Mus musculus have been found only in the major towns surveyed and along the main routes of commerce, it seems probable the infection is limited geographically and ecologically as well. Villages just a few miles from Addis Ababa (where the infection, and disease, are common) may be free of Rattus and demonstrable presence of R. mooseri in murines and ectoparasites in dwellings. Within 3 km. of Addis both Rattus and native murines may live in a domicile, but rarely share fleas. Other major findings are: 1) a sibling species of the alleged vector flea, Xenopsylla cheopis, namely X. bantonum, outnumbered it on Rattus in the endemic foci and 2)



both species of fleas were extremely abundant on indigenous murines elsewhere, in the absence of infection, and they were also common on such rodents in huts in the absence of Rattus and of R. mooseri. R. mooseri in Rattus was noted in houses in Koka town, and the rate of infection there is significantly lower than in Addis Ababa. The most common Rattus flea in Addis is Leptopsylla segnis, and this has been found to have a high rate of natural infection with R. mooseri. L. segnis has not been found in Koka, perhaps because of the higher temperature there, nor has it been found on the indigenous murines which are commensals outside of Addis, and which are infested with the allied L. aethiopica (and both species of Xenopsylla). L. aethiopica, while broadly distributed in Ethiopia, is very rare except in the highlands, where it is restricted to native murines, and where at elevations of 2600-2900 m. it may be fairly common, especially at the upper limits of altitude. It has not been found in domiciles where Rattus occurs, but may be taken on native murines indoors in the absence of Rattus. L. segnis, in contrast, is limited to Rattus and Mus and to the indoors. The two Leptopsylla may therefore exist within meters of each other, but there seems to be no cross-over. The epidemiological consequence of such factors may prove to be noteworthy. Additional data were again collected this year indicating focal differences in the infection rate of R. mooseri in Rattus. These and other points are treated in more detail below, following a discussion of the small mammals (theraphions) and ectoparasites encountered.

b. Areas and Habitats Studied

1) Six general areas have been studied thus far in this project, as cited in Table 1, which also shows the habitats therein which have been surveyed, and indicates the designation used therefor on the tables of data and in the text below. In and near Addis, 9

(TABLE 1 ABOUT HERE)

foci were being investigated, including 3 new ones, and 3 were checked in the Koka site, which was visited again in 1977. Ankober, Lemi and Menagesha proved to be of great scientific interest in 1975 and 1976 but because of the shortage of time and/or unsettled conditions, they could not be examined in 1977. The new area, Mount Intoto, just 2-4 km. from Addis, proved to be exceptionally rewarding as a study site. Intoto is a huge mountain mass immediately north of the capital, and here, merely 2-4 km. from the U.S. Embassy, we found villages of tremendous significance to our studies on murine typhus. Thus, in Intoto Kedani Mehret, the closer one, at 2600 m. elevation, Rattus co-existed in the huts with native murines like Praomys. This was the only such site known to us, and gave us an opportunity to study the possible limitation, versus cross-over, of R. mooseri infection in various rodents and their ectoparasites in a focus we were certain would prove to be endemic (as it did). The second village, Intoto Maryam, 2 km. further up the mountain, at 2900 m. was apparently free of Rattus, but Praomys etc. was abundant in the houses or were also taken therein. This provided an opportunity to compare the endemicity in 2 villages, which though close together, had important faunal differences. Intoto was remarkably free of native vegetation and in essence consists only of groves of eucalyptus, a tree introduced from Australia for timber and fuel, agricultural fields, overgrazed pasture and a large military reservation encompassing those same habitats. The only cover of indigenous or introduced herbs, bushes and trees where relict rodents were likely to be found was within the property of 2 churches at the top of the mountain (2900-2920 m.). The church grounds were sacred, and hence out-of-bounds, but we were permitted to travel along some of the garden-walls and buildings, where we found a remarkable but relict fauna of murines, a shrew, and fleas. Although we were at first welcomed on the military grounds, we were summarily banned therefrom.

c. Mammals Collected

1) Introduction

a) A total of 493 mammals were collected, examined and preserved this year, as shown in Table 2, bringing the total to 1113, representing at least 30 species.

DESIG- NATION	AREA AND HABITAT	CHART CAPTION
I.	I. ADDIS ABABA. 2300-2500m. elev.	ADDIS ABABA
I.1	1. Buildings in City. (Homes, Coffee-Shops, etc.)	ADDIS Town, Houses
I.2	2. Dwellings in Makanissa, a Suburb in SW Addis Ababa	ADDIS Suburbs, Houses
I.3	3. Makanissa Dairy Barn and Adjacent Buildings	(Ditto)
I.4	4. Scattered Huts of Furee Village, 3 km. S of Makanissa	(Ditto)
I.5	5. Agricultural Fields of Furee Village	ADDIS Suburbs, Fields
I.6	6. Makanissa Suburban Fields and Streamsides	(Ditto)
II.	II. KOKA, RIFT VALLEY. (8°35'N, 39°06'E) 100 km. SW of Addis, 1640m	KOKA-RIFT VALLEY
II.1	1. Koka Town. (Buildings Along Main Road and Street)	KOKA Town, Houses
II.2	2. Acacia Groves 5 km. S of Koka Town	Groves/Fields
II.3	3. Scattered Huts in Groves 5 km. S of Koka Town	Huts in Groves
III.	III. LEMI. (9°46'N, 38°53'E) 110 km. N of Addis, 2600m.	LEMI
III.1	1. Buildings in Town	LEMI Town, Houses
III.2	2. Along Plateau by Edge of Escarpment	LEMI, Field
III.3	3. Along Bluffs of Escarpment	(Ditto)
IV.	IV. MENAGESHA. (9°02'N, 38°35'E) 30 km. W of Addis. 2500-2850m.	MENAGESHA
IV.1	1. Buildings of St. Mary Village. 2500m.	MENAGESHA Houses
IV.2	2. In Forest Remnants etc. on Mt. Menagesha. 2850m.	MENAGESHA Field
IV.3	3. Fields near St. Mary Village. 2500m.	(Ditto)
V.	V. ANKOBER. (9°35'N, 39°45'E) 110 km. NE of Addis. 3500m.	ANKOBER
V.1	1. Overgrazed Rocky Mountain Slope	ANKOBER
VI.	VI. MOUNT INTOTO. 2-4 km. N of Addis. 2600-2920m.	INTOTO
VI.1	1. Domiciles in Intoto Kedant Mehret Village. 2km.N of Addis. 2600m.	INTOTO K.M. Domiciles
VI.2	2. Church-yards. Vicinity Intoto Maryam, 4km.N. of Addis. 2900m.	INTOTO Yards
VI.3	3. Domiciles in Intoto Maryam. 2900m.	INTOTO N. Domiciles

TABLE 1. AREAS AND HABITATS STUDIED IN MURINE TYPHUS AND SPOTTED FEVER PROJECTS IN  
 ETHIOPIA (1974-1977) -Report Page 3 -

NAME	NO. PROCESSED	
	1977	1976-77
1. <u>Crocidura</u> sp. A (Shrew) . . . . .	7	8
2. <u>Crocidura</u> sp. B . . . . .	0	1
3. <u>Crocidura</u> sp. C . . . . .	1	7
4. <u>Nycteris thebaica</u> (Bat) . . . . .	0	2
5. <u>Laephotis wintoni</u> (Bat) . . . . .	0	1
6. <u>Procavia capensis</u> (Hyrax) . . . . .	0	2
7. <u>Tachyoryctes splendens</u> (Mole-rat) . . . . .	7	14
8. <u>Graphiurus murinus</u> (Dormouse) . . . . .	0	4
9. <u>Tatera robusta</u> (Gerbil) . . . . .	0	12
10. <u>Acomys</u> sp. ? (Spiny Mouse) . . . . .	0	2
11. <u>Arvicanthis abyssinicus</u> (Grass Rat) . . . . .	34	161
12. <u>Arvicanthis dembeensis</u> (Grass Rat) . . . . .		
13. <u>Dendromus lovati</u> (Tree Mouse) . . . . .	2	12
14. <u>Dendromus</u> sp. ? . . . . .	3	4
15. <u>Desmomyys harringtoni</u> (Groove-toothed Rat) . . . . .	43	84
16. <u>Lophuromys flavopunctatus</u> (Red-bellied Mouse) . . . . .	27	43
17. <u>Mastomys natalensis</u> (Multimammate Rat) . . . . .	10	119
18. <u>Muriculus imberbis</u> (Stripe-backed Mouse) . . . . .	1	2
19. <u>Mus</u> (M.) <u>musculus</u> (House Mouse) . . . . .	16	18
20. <u>Mus</u> ( <u>Leggada</u> ) sp. (Mouse) . . . . .	20	67
21. <u>Praomys albipes</u> (Soft-furred Rat) . . . . .	118	212
22. <u>Praomys fumatus</u> . . . . .	0	16
23. <u>Praomys</u> sp. ? . . . . .	0	2
24. <u>Rattus</u> (R.) <u>rattus</u> (Commensal Rat) . . . . .	213	308
25. <u>Stenocephalemys albicaudata</u> (Ethiopian Narrow-headed Rat) . . . . .	6	6
26. <u>Otomys typus</u> (Swamp Rat) . . . . .	2	2
27. <u>Lepus capensis</u> (Cape Hare) . . . . .	1	1
28. <u>Herpestes ichneumon</u> (Mongoose) . . . . .	2	3
29. <u>Genetta tigrina</u> (Genet) . . . . .	2	2
30. <u>Ictonyx striatus</u> (Skunk) . . . . .	2	2
TOTALS	493	1113

TABLE 2. LIST OF MAMMALS COLLECTED IN ETHIOPIA, 1976-1977.

Of these, #10-#26 are murids. Some of these theraphions (small mammals) may be new to Science, at least at the subspecies level. Notably, some of the most common murines are extremely difficult for even the specialist to identify because of inadequate knowledge about the classification of rats. Genera like Arvicanthis, Praomys, Mastomys, Desmomyys and Mus in particular are in need of review, and our specimens are of value as museum material for that reason. Even the status of Rattus rattus in Ethiopia is in doubt for there are 2 distinct forms of the rats occurring together in buildings - a white-bellied one and a dark-bellied one, and there is controversy as to whether these represent different subspecies, or even species, or if they are color varieties of a single taxon of rat, which vary consistently in other features as well.

#### 2) Notes on Important Mammals

a) The relevant distribution of the major species of rodents involved in our study is indicated in Tables 5, 6 and 8 below, and the differences exhibited are obviously important factors in the ecology of murine typhus and probably spotted fever as well. This year we concentrated on Rattus because the evidence to date clearly indicated that this taxon is the



most critical mammalian factor in the ecology of murine typhus in Ethiopia. This entailed intensive trapping in domiciles, which meant we would be getting very few indigenous mammals. Another reason for the lessened number of species and individuals of theraphions collected in 1977 was that the Ethiopian Government suddenly prohibited us from working outside of Addis. Intoto was technically within the limits of Addis, or so we argued. Some of the murids and rodents taken are considered briefly below.

b) Rattus. The most striking and significant point is that Rattus has a very limited distribution, and was collected only in or near Addis Ababa and at Koka. Further, within those areas, virtually all the Rattus were taken only in buildings, the single exception in both 1975 and 1976 was one rat in the Koka groves (Habitat II.2 on Table 1). We have collected several thousand murines in the fields around Koka, Addis and elsewhere in Ethiopia since 1973 and have never trapped rats away from the walls of a building on any other occasions, even when there was heavy vegetative cover a few feet distant. However, since Rattus rattus occurs in huts scattered in the fields of Koka (II.3) and suburbs of Addis (I.4), it seems likely that some individuals of this introduced species of rat may penetrate such dwellings after traversing agricultural or pastoral areas en route from nearby towns along the main roads, although many must be brought into huts along with man's effects. In the huts in the middle of the fields of Koka and Addis suburbs, the Rattus characteristically lived in the roofs and were most readily trapped on high shelves. The ground level of the huts in the Koka fields were infested with Mastomys and Arvicanthis, and the buildings were so poorly constructed that runways of the murines could be readily discerned entering and leaving dwellings, with burrow-openings indoors.

c) We previously reported that, to our surprise, we failed to collect Rattus in domiciles and buildings of Menagesha (Area IV), which not only is merely 30 km. from Addis, but the village in question is within 1 km. of the main road north from the capital. These rats were also apparently absent from Leml (III), which is about 40 km. from the main road. These findings suggest that Rattus is a recent entry into Ethiopia and that these rats have not yet had time to establish themselves in the fields and in domiciles removed from the main routes of commerce. It may be argued that their restricted range in Ethiopia is due to climate, i.e. Addis, at 2500 m. elev., is definitely cold at night throughout the year, and hence the Rattus live only indoors, where it is warmer, even in dwellings of poor people. The impact of this argument is heightened by the absence of Rattus at Ankober (V), where it is near or below freezing at night. Temperature no doubt is an important factor affecting the distribution of Rattus rattus, but it alone could not explain the absence of Rattus in the fields of Koka, in the Rift Valley, where the daytime temperature reaches 100° F. and that at night is mildly temperate. Aridity must be considered as a limiting factor for Rattus in the Rift Valley, for murids in general (but with marked exceptions) and Rattus in particular are rare or absent in xeric areas. However, our study site was along the shores of a lake, and some of the fields were irrigated, and besides, there is a definite rainy season of weeks or months in the area. The factors of temperature and humidity could not satisfactorily explain the absence of Rattus in the field and buildings at Leml and Menagesha, especially when one considers that Rattus is abundant in the agricultural and other outdoor areas of Kenya etc. (Heisch et al., 1962; Heisch, 1969). However, as reported by Hopkins in 1949, Rattus was "hardly ever encountered in Uganda outside buildings" (p. 39) and the 5 exceptions encountered in the 5-year study (1929-1934) were in close proximity to buildings, suggesting the current situation in Ethiopia. Hopkins also mentioned that while Mastomys ("Rattus coucha") co-existed with Rattus rattus in some huts and areas, the latter eventually ousted the Mastomys completely from buildings. He regarded Rattus as having penetrated up-country Uganda after having entered in the early 20th century via sea-ports, although Schwarz (1935) claimed the route of entry was overland from the Nile Valley. Heisch et al. (1953) reported similar findings for Rattus in Kenya, stating that this species



was restricted to buildings or in the immediate vicinity thereof and was not found in the fields. In that country Rattus was found in burrows in the earth floor, and not in the roofs.

d) As mentioned above, in 1977 we made the important observation that just 4 km. from Addis, on Mt. Intoto, there apparently were no rats in the villages at 2900 m. (Intoto Maryam), but lower down, at 2400 m. (Intoto Kedani Mehrat), merely 2 km. from Addis, there were Rattus in the huts, together with native murines like Praomys, Desmomys and Lophuromys. The absence of Rattus at the higher elevation, where these same indigenous murines were common, along with an occasional Mus suggest to us that cold temperature is indeed the limiting factor for Rattus in the Ethiopian highlands. These points about the distribution, peregrinations and length of inhabitation of Ethiopia are all of epidemiological significance, for as indicated below, the evidence is that Rattus is the critical factor in the ecology of the infection, at least in that country.

e) House Mice. Mus (M.) musculus and the Subgenus Leggada. Since M. musculus has been implicated in the epidemiology of murine typhus in eastern Europe (Petrov, 1940), China (Liu & Zia, 1940, 1941), Texas (Keaton et al., 1953) and, on theoretical but logical, grounds in Australia (Derrick & Pope, 1960), they are relevant to our program in Ethiopia. House mice occur in the Addis area, and may be common in houses there and elsewhere, but since we have had to concentrate on Rattus and rat-sized murines in Addis town and suburbs, the live-traps employed have been too large to collect adequate numbers of house-mice to provide optimal information about the incidence and distribution of infection with R. mooseri, etc. We know that Mus does occur as high as 2900 m., at Intoto Maryam, although it is apparently scarce there. Small traps, used for Praomys and hence suitable for Mus produced 80 of the former but only 1 of the latter. Unquestionably, Mus of the subgenus Leggada are common in all the habitats surveyed outdoors thus far, at least where there is a modicum of vegetative cover.

f) Arvicanthis ("Grass Rats"). Native rats of the murine genus Arvicanthis are of special interest to us since we believe that the true and original host of X. cheopis must be an Arvicanthis in the lower elevations of northern Africa, and since that species of flea is extremely prevalent on grass rats in the dry season in the Rift Valley. Further, in 1975 and '76 we reported natural infection with rickettsiae of the spotted fever group in Arvicanthis at Koka, and in their ticks. Apparently we have collected at least three species of Arvicanthis, but they have not yet been precisely identified. All were diurnal and highly abundant within their particular habitats, and presumably dominant therein. One specimen was collected at Koka; another in the fields near Addis; at least one at the Ankober site. Arvicanthis were plentiful at Lemi, Menagesha, and at Intoto, and there may be more than one species involved. In the literature, and in earlier collections, "Arvicanthis" may have been confused with "Desmomys". Arvicanthis was commonly observed in huts scattered in the fields of Koka. Similar habits were noted for this genus in Uganda (Hopkins, 1949) and Kenya (H. Hoogstraal, in litt.).

g) Other Native Murines. The multimammate rat, Mastomys natalensis was plentiful at Koka and in the suburbs of Addis as well as at Lemi and Menagesha. We did not collect it at Intoto, and believe it is too cold there for this species. At Koka Mastomys occupied the same runs as Arvicanthis, but was primarily nocturnal. This rat is considered to be a commensal species, as well as a field rat, in many parts of Africa, and such trait has been observed in our Ethiopian studies at Lemi and Menagesha. The same was found to be true for Praomys albipes in those two areas, and at both Intoto sites, where it was the predominant rat in domiciles. In fact, in those sites, and in the huts in the Koka fields, the local people complained bitterly about depredations and incursions of these native rats (Praomys at the higher elevations, Mastomys at Koka), and claimed they were often bitten or had their hair licked by rats while in bed. Another indigenous murine which proved capable of living in buildings when given the opportunity was Desmomys, at Lemi, Menagesha and both Intoto villages. Species of Lophuromys are found in

many parts of Africa, and prior to this year we had encountered the genus in Ethiopia only in the mountains. Finding it at Lemi, where the elevation is lower than at Addis was therefore unexpected, but it may be that the main criterion for its presence is rocky terrain. It was present in the huts on Intoto (both villages) and was abundant in there gardens at 2900m. here. As at Ankober, Lophuromys was active on Intoto during the daytime as well as at night (like Otomys, which we had trapped at Ankober and Intoto Maryam). Stenocephalemys was also taken at the top of Intoto, and must be a high montane form.

h) Other Theraphions. This year we did not trap intensively in the outdoor habitat in which gerbils occurred, i.e. at Koka. The rhizomyid, burrowing, mole-rat Tachyoryctes splendens which was common in the Menagesha area, was collected in the flat parts of Intoto at 2900m. A large, commensal Crocidura was collected in huts in the Koka groves (II.2), as at Lemi (III.1) previously. This is probably the shrew reported as Suncus previously because of its unusual size and is of special interest because in 1975 some of its ticks, at Koka, were naturally infected with rickettsiae of the spotted fever group.

d. Ectoparasites

1) Fleas

a) Xenopsylla. (1) According to the consensus of informed opinion, X. cheopis is supposed to be the most important vector of murine typhus, and it is therefore especially notable that wherever we collected this species, it was accompanied (on Rattus, Mastomys etc.) and often outnumbered by a sibling species, X. bantorum, even in Addis town. Nothing was known about the possible role of this latter species in the ecology of murine typhus, but as noted below, its rate of natural infection with R. mooseri is comparable to that of X. cheopis. Both species occurred in the absence of Rattus, and in fact were much more common on the native hosts than on Rattus. This is shown in Table 3, which deals with the "index", the average number of fleas per individual host in the various study areas.

(TABLE 3 ABOUT HERE)

(2) In Addis Ababa, the X. cheopis index on Rattus in 1977 was 0.3, the same as last year, but that for X. bantorum was more than double that for X. cheopis and also much higher than that in 1976. In the suburbs the index for each species was twice as high as in town in 1977, but even that meant the average per Rattus rat was well below 1. In Koka, however, the Xenopsylla index was very much lower in 1977 than in 1976. Thus, the figures show a 13-fold or greater reduction for each species on Rattus in town, and a 4-10-fold reduction on field rodents such as Mastomys and Arvicanthis which we believe are the native hosts for X. bantorum and X. cheopis respectively. This marked drop in numbers at Koka is ascribed to the late occurrence of the rainy season in the area. It will be recalled that the populations of Xenopsylla there are 100 times greater in the dry season than at the height of the rains and immediately thereafter. We had reported on such seasonal changes in Malaya and Pakistan (Traub, 1972D), although such variations had not been noted in the literature on plague. The heavy recent rains at Koka had resulted in flooding of the Acacia groves and most of the Arvicanthis and other rodents there had apparently either drowned or fled, since they were remarkably uncommon instead of visibly swarming, as in the past.

(3) At Intoto, X. cheopis and X. bantorum on Rattus were more common than in the other areas mentioned above, but it is stressed that these rats were only found indoors and at the lower village only. These fleas, however, also occurred on native murines at Intoto (Table 4) and actually ranged as high as 2900 m., both on Fraomys and Desmomyss in houses and even on Lophuromys outdoors, with an index comparable to that observed for Rattus in Addis. Xenopsylla seems to be near the limit of its range in the cold weather outdoors on Intoto, as suggested both by its low index when found and its absence on outdoor hosts like Stenocephalemys, Otomys, etc. Much of the rest of the Addis area is at the same elevation as Intoto, and we remarked last year on the extreme dearth of X. cheopis on field-rodents there. In 1976 we

	RATTUS		MASTOMYS		ARVICANTHIS		MUS		MUS		CROCIDURA	
	RATTUS		NATALENSIS				MUSCULUS		(LEGGADA)			
	'76	'77	'76	'77	'76	'77	'76	'77	'76	'77	'76	'77
I. ADDIS ABABA												
A. TOWN. HOUSES			X		X		@		X		(?)	
X. CHEOPIS	0.3	0.3					+	+				
X. BANTORUM	0.4	0.7					+	+				
B. SUBURBS. 1. BUILDINGS			-		X		X	@	X		(?)	
X. CHEOPIS	0.27	0.6	X					+				
X. BANTORUM	0.25	1.4						+				
B. SUBURBS. 2. FIELD	X		@		@		X		@		@	
X. CHEOPIS			R	R	0	0			0	0	0	0
X. BANTORUM			R	R	0	0			0	0	0	0
II. KOKA												
A. TOWN. HOUSES			(?)		X		X		X		(?)	
X. CHEOPIS	2.0	0.15										
X. BANTORUM	3.0	0.15										
B. ACACIA GROVE	R	X					X		@		@	
X. CHEOPIS			4	1	50	5			0	0	0	0
X. BANTORUM			20	4	6	1			0	0	0	0
C. HUTS IN FIELD							X					
X. CHEOPIS	2.0	0.1	2.5	0	60	6					3.0	9.0
X. BANTORUM	3.0	0.2	3.5	0	7	1					3.0	12.0
III. MT. INTOTO												
A. I. K. MEHRET. HOUSES				X		X			X		(?)	
X. CHEOPIS		0.20						0				
X. BANTORUM		0.24						0				
B. I. MARYAM. HOUSES		X		X					X		(?)	
X. CHEOPIS						0		0				
X. BANTORUM						0		0				
C. I. MARYAM. OUTDOORS		X		X				X				
X. CHEOPIS						0			0		0	0
X. BANTORUM						0			0		0	0

TABLE 3. XENOPSYLLA CHEOPIS AND X. BANTORUM IN VARIOUS AREAS AND HABITATS IN ETHIOPIA. THE "INDEX" ON RATTUS, MASTOMYS AND OTHER MAJOR HOSTS. (1976-1977)

Numbers = "Flea Index" = average number of fleas per individual host.  
 R = Host or flea rare. + = Index less than 0.1. @ = Host present and may be common. X = Host not collected. 0 = Flea not collected.  
 (?) = No data but believed present.



	PRAOMYS ALBIPES (1977)	DESMOMYS HARRINGTONI (1977)	LOPHUROMYS FLAVO- PUNCTATUS (1977)	STENO- CEPHALEMYS (1977)	OTOMYS & TACHYORYCTES (1977)	TATERA ROBUSTA (1976)
I. ADDIS ABABA AREA						
A. ANY BUILDINGS XENOPSYLLA	X	X	X	X	X	X
B. FIELDS XENOPSYLLA	X	X	X	X	X	R
II. KOKA						
A. ANY BUILDINGS XENOPSYLLA	X	X	X	X	X	X
B. ACACIA GROVES X. NILOTICA only	X	X	X	X	X	10.0
III. MT. INTOTO						
A. I.K. MEHRET, HOUSES				X	X	X
X. CHEOPIS	0.11	0	0			
X. BANTORUM	0.22	0	0			
B. I. MARYAM, HOUSES				X	X	X
X. CHEOPIS	0.15	0.1	0			
X. BANTORUM	0.18	0.2	0.2			
C. I. MARYAM. OUTDOORS						X
X. CHEOPIS	+	0	0	0	0	
X. BANTORUM	+	0	0.2	0	0	

TABLE 4. XENOPSYLLA CHEOPIS AND X. BANTORUM IN VARIOUS AREAS AND HABITATS IN ETHIOPIA. THE INDEX ON PRAOMYS, DESMOMYS AND CERTAIN OTHER HOSTS (1976 or 1977)

Numbers = "Flea Index" = average number of fleas per individual host.  
 R = Host or flea rare. + = Index less than 0.1. X = Host not collected. 0 = Flea not collected.



also pointed out that both of these Xenopsylla were found on native murines in houses at Lemi and Menagesha, in the absence of Rattus, and were more common there than on Addis Rattus. Notably, in those two areas, X. cheopis and X. bantorum were far more abundant on domiciliary murines than on the field-rodents (which included the same species of Praomys, Desmomys etc.). In general, then, the data suggest that in the highlands present rigorous conditions for survival of both Rattus and Xenopsylla. This is borne out by failure to collect either this host or genus of flea in 1975 in the frigid climes of Ankober.

(4) The low indices of X. cheopis and X. bantorum on Rattus in the Addis area warrant comment, and further study. Since many of the rats in Addis town, and particularly in the suburbs, lacked Xenopsylla altogether, while an occasional one had 5-10 such fleas, it might be thought that the collecting techniques account for the discrepancies. This is unlikely, however, because 1) these same rats had an over-all index of 7.5-9 for L. segnis. 2) Mastomys and Arvicanthis, and even Rattus collected elsewhere had substantially higher indices, as indicated above. It is possible that Xenopsylla tend to leave the hosts readily when the animals become agitated in the traps, and that the occasional Rattus with a large number of Xenopsylla had been freshly trapped, but the same factor should apply elsewhere and can only be of relative importance. There is also a dearth of species of Xenopsylla in the Ethiopian highlands, and not only of numbers of individuals. X. cheopis and X. bantorum are the only species collected at the sites above 2300 m. in elevation, although a third species X. nilotica, associated with Tatera at Koka, probably occurs, together with that host, in certain habitats at the level of Addis. Low temperatures, then, seem to be a major factor, to which must also be added the prolonged period (6 months) of heavy rains characteristic of the area.

(5) The importance of highly localized conditions as affecting the abundance of fleas, and in skewing a figure like the flea-index, is shown by the data on 2 Crocidura from a particular native hut in the fields of Koka. These shrews had an index of 20 and 23 Xenopsylla respectively, with X. bantorum slightly outnumbering X. cheopis, in an area and at a time when the index of these fleas on their true hosts outdoors was only between 1 and 5. Crocidura is not an especially good host for Xenopsylla, (i.e. the X. cheopis index in 1976 was the same as that on Mastomys in huts and 1/20 that of such Arvicanthis) so the difference in numbers is probably due to environmental factors in the breeding-sites of the fleas.

b) Leptopsylla. (1) The so-called house-mouse flea, L. segnis occurs on rats in various parts of the world, and may, indeed, turn out to be a characteristic flea of Rattus rattus. In Ethiopia, wherever this species occurred, it was far more prevalent on Rattus than were either species of Xenopsylla, as can be seen by comparing Table 5 (on Leptopsylla) with Table 3. Moreover, L. segnis is virtually restricted to Rattus, even when there are other murines coexisting in the domiciles (Table 5 and 6). These may be important points in the ecology of murine typhus, and are discussed further here (and below, vide p. 33 of this Report).

(2) The specificity of L. segnis is striking. Virtually all the records are from Rattus or Mus musculus, the only exceptions being an occasional specimen from Praomys living in the same house, and even here the infestation rate was not more than 1 flea on 10 Praomys, whereas the average rat in the house carried 7.2 L. segnis. In the Addis suburbs, the index on Rattus was 9 and some individuals were infested with 42, or even 52 L. segnis. Such high infestations of course skew the index, but this is somewhat balanced by the fact that fleas tend to leave a rat that is agitated and hyperactive in a trap for hours before being caught. Rats examined shortly after being trapped tend to have much higher indices than those overnight in a trap. L. segnis is essentially a sessile flea, remaining hooked onto the hairs of the host by special spiniforms on the frontal margin of the head of the flea, concentrated in a group on the muzzle or crown of the rat (Traub\*, 1977). This is best observed in freshly-caught hosts, whereas the fleas tend to be loose and scattered on excited rats, an observation that supports the above contention.

(3) Other important points about L. segnis are that 1) it was never collected at Koka (presumably because it is too hot and arid there); 2) it was 7-30 times as abundant as Xenopsylla on rats where the two taxa co-exist, and 3) while L. segnis has been found by us in areas where Rattus was not collected, this has only been in foci where Mus musculus occurred, e.g. Intoto Maryam. The potential epidemiological significance of these sundry observations is discussed below (Report p. 33; Application p. 66).

(4) L. aethiopica is a characteristic parasite on Mus (Leggada) and insofar as our own observations are concerned, has been taken on that species whenever it occurs. Since (Leggada) has been trapped in every area under study, this means that L. aethiopica is equally widely distributed, and is the only species of flea that has so broad a range. It is seldom collected, probably because it leaves its host soon after death or capture, but even freshly caught Mus (Leggada) frequently lack fleas. When infested, the usual L. aethiopica index is about 4. As shown in Table 5 and 6, this species was relatively common on Praomys in domiciles (TABLE 5 and 6 ABOUT HERE)

on Mt. Intoto and at Intoto Maryam on Lophuromys, but was seldom collected on the native murines outdoors. L. aethiopica was abundant (an index of 4) on such hosts in buildings in Lemi and Menagesha in 1976. In general, indoors in the highlands, this species was more common on murines like Praomys and Mastomys than X. cheopis and X. bantonum were on Rattus. However, the only place where the Xenopsylla, Rattus and Praomys, Desmomyss co-existed were in buildings at Intoto Kedani Mehret. Here (and elsewhere) the Rattus were never found infested with L. aethiopica even if it was present on Praomys. There was then, a surprising lack of exchange of "specific" fleas like Leptopsylla between Rattus and the native murines even in the same domicile. This is further borne out by the data on indigenous fleas like Ctenophthalmus n. sp., Chiastopsylla n. sp. etc. which, as mentioned below, were common on indoor Praomys, Desmomyss and Lophuromys on Intoto. Only one specimen of such a flea was collected on Rattus. In contrast, catholic species like Xenopsylla infested the native murines as readily as Rattus in domiciles at I.K. Mehret, and freely parasitize Praomys, Desmomyss etc. in areas where Rattus is absent. This suggests that the barrier between Rattus and the native murines indoors is not purely a physical one keeping their fleas apart, such as if the Rattus were restricted to the roofs, and their fleas could only breed in thatch, etc. there, while the native murines and their fleas lived in burrows in the floor of the huts. We believe that to a large degree there is little physical contact between Rattus and the native murines, as mentioned below (Report p. 33 ; Application p. 66 ), but host-specificity and not just lack of opportunity plays an important role in minimizing "exchange" of fleas in the huts.

c) Ctenophthalmus. (1) Fleas of the genus Ctenophthalmus are the dominant members of the rodent fauna in the Ethiopian highlands (i.e., all surveyed areas save Koka), both as concerns numbers of specimens and of species. Freshly caught hosts frequently had an index of 6-10 Ctenophthalmus. At least 10 species new to Science are represented in the collections to date, and these are of great interest from the aspects of taxonomy, evolution and zoogeography. Most of the species are fairly host-specific. The zoogeographic implications are discussed below (Report p. 39; Application p. 72).

d) Dinopsyllus. (1) The Dinopsyllus lypus complex has been found in all areas under study. D. lypus is found throughout much of Africa south of the Sahara, and infesting a variety of hosts, primarily murines. This species (under various names) is of consequence as a vector of plague, at least among rodents (de Meillon et al., 1961; Pollitzer, 1954). Although some of the Ethiopian native rats carried 3-4 Dinopsyllus, in general this taxon was uncommon and had an over-all index of about 0.30. A few specimens of a new species of a giant Dinopsyllus were collected at Ankober, on Stenocephalemys, in 1975, and the highland species include several siblings of D. lypus that are new to Science.

e) Xiphopsylla. (1) This genus consists of a group of little-known species in a distinctive family. Three new species were collected from Otomys and Stenocephalemys at

	RATTUS RATTUS		MASTOMYS NATALENSIS		ARVICANTHIS		MUS MUSCULUS		MUS (LEGGADA)		PRAOMYS ALBOPICTUS	
	'76	'77	'76	'77	'76	'77	'76	'77	'76	'77	'76	'77
I. ADDIS ABABA												
A. TOWN. HOUSES				X		X		@		X		X
L. SEGNIS	5.3	6.7					5	6				
L. AETHIOPICA	0						0	0				
B. SUBURBS. 1. BUILDINGS				X		X	X	@				X
L. SEGNIS	7.0	9.0						2	0	0		
L. AETHIOPICA	0	0						0				
B. SUBURBS. 2. FIELD		X						X				X
L. SEGNIS			0	0	0	0			0	0		
L. AETHIOPICA			0	0	0	0			0.4	0.5		
II. KOKA												
A. HOUSES OR HUTS												X
L. SEGNIS	0	0	0	0	0	0	0	0	0	0		
L. AETHIOPICA	0	0	0	0	0	0	0	0	0	0		
B. ACACIA GROVE	R	X						X				X
L. SEGNIS	0	0	0	0	0	0			0	0		
L. AETHIOPICA	0	0	0	0	0	0			+	+		
III. MT. INTOTO												
A. I.K. MEHRET. HOUSES				X		X						
L. SEGNIS		7.2										0.1
L. AETHIOPICA		0										1.4
B. I. MARYAM. HOUSES		X		X					X			
L. SEGNIS						0		0.3				+
L. AETHIOPICA						0		0				1.9
C. I. MARYAM. OUTDOORS		X		X				X				
L. SEGNIS										0		0
L. AETHIOPICA										0.5		0.8

TABLE 5. LEPTOPSYLLA SEGNIS AND L. AETHIOPICA IN VARIOUS AREAS AND HABITATS IN ETHIOPIA. THE INDEX ON RATTUS, MASTOMYS, PRAOMYS AND CERTAIN OTHER HOSTS. (1976 or 1977)

Numbers = "Flea Index" = average number of fleas per individual host.  
 R = Host or flea rare. + = Index less than 0.1. @ = Host present  
 and may be common. X = Host not collected. 0 = Flea not collected.



	DESMOMYS HARRINGTONI (1977)	LOPHUROMYS FLAVO- PUNCTATUS (1977)	STENOCEPH- ALEMYS (1977)	OTOMYS AND TACHYORYCTES (1977)	TATERA ROBUSTA (1975)	CROCIDURA (1975-77)
I. ADDIS ABABA AREA						
A. ANY BUILDINGS LEPTOPSYLLA	X	X	X	X	X	(?)
B. FIELDS LEPTOPSYLLA	X	X	X	X	0	0
II. KOKA						
A. ANY BUILDINGS LEPTOPSYLLA	X	X	X	X	X	0
B. ACACIA GROVES LEPTOPSYLLA	X	X	X	X	0	0
III. MT. INTOTO						
A. I. K. MEHRET. HOUSES			X	X	X	(?)
L. SEGNIS	0	0				
L. AETHIOPICA	0	0				
B. I. MARYAM. HOUSES			X	X	X	(?)
L. SEGNIS	0	0				
L. AETHIOPICA	0.25	2.0				
C. I. MARYAM. OUTDOORS					X	
L. SEGNIS	0	0	0	0		0
L. AETHIOPICA	0.17	0	+	0		0

TABLE 6. LEPTOPSYLLA SEGNIS AND L. AETHIOPICA IN VARIOUS AREAS AND HABITATS IN ETHIOPIA. THE INDEX ON DESMOMYS, LOPHUROMYS AND CERTAIN OTHER HOSTS (1975 or '76 or '77)

Numbers = "Flea Index" = average number of fleas per individual host.  
 + = Index less than 0.1. X = Host not collected. 0 = Flea not collected. (?) = No data but believed present.



Ankober in 1975, and as in the case of the montane Ctenophthalmus, the affinities are with the Xiphopsylla from the mountains of East Africa. One of these was collected at Intoto this year, but its present status has not yet been determined.

f) Chiaestopsylla. (1) The family to which the genus Chiaestopsylla belongs has never heretofore been found north of the Congo region, and the genus Chiaestopsylla has been referred to as: "peculiar to Southern Africa" (de Meillon et al., 1961) and has never been found north of Rhodesia. Our finding of 2 new species of Chiaestopsylla at Lemli (and one of these also at Menagesha) was therefore of real interest, particularly since murines in the houses were at times infested with these fleas. Some South African Chiaestopsylla are regarded as intramurid vectors of plague but it doesn't seem to be known if the species bite man. Both of these new species were collected at Intoto this year, and some specimens were taken on native rodents in huts. The fact that these species are commonly found on native rats in domiciles may prove to be of medical significance.

g) Echidnophaga. (1) Sticktight fleas of the genus Echidnophaga i.e., E. gallinacea, have been found naturally infected with R. mooseri and also capable of experimental transmission of the agent (Brigham, 1941; Alicata, 1942) but their importance as vectors has been belittled because of the poor correlation between the distribution of the flea and that of the hosts (Cole & Koepke, 1946). Rats in the houses in Koka were again found to be commonly infested with many specimens of a new and hypodermal species of Echidnophaga. The species has not been encountered elsewhere, at least thus far. In view of its host, habits and occurrence in dwellings, this Echidnophaga is worthy of study.

h) Cosmopolitan or Introduced Fleas. (1) The cat-flea, Ctenocephalides felis, was collected at Koka and Addis on such hosts as mongoose, hare, dog, etc. This species is suspected as having been a source of murine typhus infection in Man (Adams et al., 1970, Irons et al., 1944, 1946; Keaton et al., 1953). Pulex irritans, which usually infests large animals like man and pigs, was found on Rattus in Addis, and once 9 fleas were collected from one host, an unusual and potentially significant record. This flea, which readily bites man (as the name "human flea" implies) has never been seriously investigated as a possible vector of murine typhus. It is apparently fairly common in Addis.

## 2) Other Ectoparasites

a) Lice. (1) Reasons have been advanced previously for the need to study lice as possible vectors of R. mooseri, and the findings reported then and below, on natural infection in Ethiopian lice from Rattus (Rept. p.27, Appl. p. 60) emphasize this point. Two species of lice, namely Polyplax spinulosa and Hoplopleura oenomydis have been found on Addis Rattus, but generally diligent search of the hosts is necessary to discover them, and data are yet unavailable on their relative or absolute numbers, although P. spinulosa seem to outnumber H. oenomydis in our collections. Identifications of the lice collected on other murines have not yet been completed.

b) Chiggers. (1) In certain areas and seasons, it is easy to collect chiggers (trombiculid mites) because they occur in large patches on the ears or other parts of the body of the host. Under other circumstances, diligent search, individual handling or special techniques are required to collect them. During our field-work in Ethiopia, collecting chiggers was in the latter category, and because of the tremendous work-load of other duties, it was impossible to do the job properly. Nevertheless, the material we have collected has proven to be of great interest and suggests that serious attention should be paid to Ethiopian chiggers from the points of view of potential vectors of disease, taxonomy, and zoogeography.

(2) In 1975 we reported that for the first time, an African Leptotrombidium had been found which is related to the vector of chigger-borne rickettsiosis. The implications of this discovery are mentioned below, under "Other Rickettsial Infections" (Rept. p.39 Appl. p.72). This Leptotrombidium, a new species in the subgenus Leptotrombidium, resembles one we had described from the Himalayas (L. irregulare), and is likewise a montane form, having been

collected on a variety of murines at Ankober. It represents the first true Leptotrombidium (Leptotrombidium) reported in Africa. A second such new member of the subgenus was collected in 1976, and the list of chiggers taken by us in Ethiopia prior to the 1977 trip is as follows:

<u>Leptotrombidium</u> ( <u>Leptotrombidium</u> ) n.sp. #1	<u>Afracarus</u> ( <u>Arabacarus</u> ) n.sp.
<u>Leptotrombidium</u> ( <u>Leptotrombidium</u> ) n.sp. #2	<u>Gahrlepiea</u> n.sp.
<u>Leptotrombidium</u> ( <u>Hypotrombidium</u> ) n.sp.	<u>Gahrlepiea nana</u>
<u>Leptotrombidium</u> ( <u>Ericotrombidium</u> ) n.sp.	<u>Rudnicola knighti</u>
<u>Microtrombicula</u> n.sp.	<u>Odontacarus</u> n.sp.
<u>Microtrombicula mastomyia</u>	
<u>Microtrombicula abyssinica</u>	
<u>Microtrombicula agnoi</u>	

Since no fewer than 8 of the 13 species listed are new to Science, it is obvious that the trombiculid fauna of Ethiopia is in need of study. When the particulars on the 1977 collections become available, the list of new and known species will probably be extended.

c) Ticks. (1) It should be noted that since in this study we are primarily dealing with rodents, we generally collect larval (seed) ticks, and only occasionally, nymphs (which parasitize medium-sized hosts), and rarely adults (which infest mainly large hosts like carnivores and cattle). Further, the immature stages are difficult or impossible to identify to species.

(2) The ticks we have collected thus far in this project have proven to be of marked interest, particularly since ticks infected with rickettsiae of the spotted fever group were found in several of the study areas. This was the case again this year (Rept. p. 28, Appl. p. 61), but the 1977 ticks have not yet been identified. It is stressed that some of the ticks and/or native murines infected with these rickettsiae were actually collected in the huts of the native people, as at Koka and Intoto. An incredibly high proportion of the rodents at Koka have evidence of infection with spotted fever rickettsiae, indicating that infected ticks must be common there. Having ticks and native rodents in the homes must be a real hazard to health in many parts of Ethiopia.

d) Mesostigmatid Mites. (1) These "blood-sucking mites" were present on the theraphions in all the areas studied, and on most species, but in general were noted only in small numbers. In 1975 we reported, on the basis of the direct FA test, that mites from a shrew at Koka were infected with rickettsiae of the spotted fever group, and we have similar data this year for 4 mites from Desmomyia in a hut on Mt. Intoto. Despite their known and potential importance as vectors in various parts of the world, this group of mites is being neglected nowadays, outside of the USSR, and thus far we have been unable to locate a scientist who is both qualified and willing to identify our Ethiopian mesostigmatid mites.

e. Serological Data on Murine Typhus Infection in Rodents

1) Evaluation of Methodology. In 1976 we reported that we had checked on the validity of the indirect fluorescent antibody (IFA) tests; as per the techniques we had developed, by checking the samples with the microagglutination (MA) test, and had reported a virtually perfect correlation in the results. We then compared the results of using filter paper discs with those obtained with sera, and made independent checks in each case with both the MA test and another set employing a complement fixation (CF) test using murine typhus specific antigens. As reported in 1976, there was a high-perfect correlation. We therefore have full confidence in the IFA test as employed here, and its sensitivity is amply demonstrated in the results cited below.

2) Results of the IFA Test for Murine Typhus. a) Table 7 and 8 show the results of the IFA tests for R. mooseri infection, for rodent blood, collected and dried on filter paper

(TABLES 7 and 8 ABOUT HERE)

	RATTUS RATTUS	MUS MUSCULUS	MUS (LEG- GADA)	PRAOMYS ALBIPES	ARVI- CANTHIS SP.	DESMOMYS HARRING- TONI	SUBTOTALS
COMMENSAL	100%	100%	NO	PARTLY	PARTLY	PARTLY	
<u>ADDIS ABABA</u>							
TOWN. BLDGS. '75-76	20/41 =49%	@	X	X	X	X	20/41 =49%
TOWN. BLDGS. '77	32/51 =63%	@	X	X	X	X	32/51 =63%
SUBURBS. BARN '75-76	19/22 =86%	@	X	X	X	X	19/22 =86%
SUBURBS. BARN '77	22/29 =76%	1/7=14%	X	X	X	X	23/36 =64%
SUBURBS. HOMES '75-76	25/47 =53%	@	X	X	X	X	25/47 =53%
SUBURBS. FIELDS '75-76	X	X	0/4	X	0/49	X	0/53
<u>KOKA</u>							
TOWN. BLDGS. '76	3/18 =17%	(?)	X	X	X	X	3/18 =17%
TOWN. BLDGS. '77	0/80	(?)	X	X	X	X	0/80
FIELDS '75-76	0/8	X	@	X	0/92	X	0/100
	(7 in Huts)						
FIELDS '77	X	X	@	X	0/7	X	0/7
LEMI. '76	X	@	@	0/28**	0/10	0/5*	0/43
MENAGESHA. '76	X	@	@	0/19**	@	0/3*	0/22
ANKOBER. '75	X	(?)	@	X	0/17	(?)	0/17
<u>MT. INTOTO '77</u>							
KEDANI MEHRET. HOMES	13/48 =27%	0/1	X	1/38 = 3%	X	0/8	14/95 =16%
MARYAM. HOMES	X	0/1	X	2/80=2.5%	@	0/5	2/86 = 2%
MARYAM. OUTDOORS	X	@	@	@	0/9	0/6	0/15
SUBTOTALS							
1975-76	67/136=49%		0/4	0/47	0/168	0/8	67/363=18%
1977	67/208=32%	1/9=11%		3/118=2.5%	0/16	0/19	71/369=19%
TOTALS							
1975-77	134/344=39%	1/9=11%	0/4	3/165=2%	0/184	0/27	138/733=19%

TABLE 7. RESULTS OF 733 INDIRECT FLUORESCENT ANTIBODY TESTS FOR MURINE TYPHUS  
 (R. MOOSERI) INFECTION FOR RATTUS, MUS AND 3 OTHER MURINES IN SPECIFIED  
 AREAS AND HABITATS IN ETHIOPIA (1975-1977)

Numerator = Number positive. Denominator = Number tested. X = Rodent not  
 collected and may be absent. @ = Rodent present but not tested. (?) =  
 Not collected, and probably present. \* = Tested with fluorescein-conjugated  
 antiserum to Arvicanthus globulin. \*\* = Ditto re. Mastomys.



	LOPHUROMYS FLAVJ- PUNCTATUS	MASTOMYS NATALENSIS	OTOMYS TYPUS	STENO- CEPHAL- EMYS	TACHYO- RYCTES SPLENDENS	TATERA ROBUSTA	SUB- TOTALS	GRAND TOTALS (INCLUDES TABLE 4)
COMMENSAL	PARTLY	PARTLY	NO	NO	NO	NO		
<u>ADDIS ABABA</u>								
TOWN.BLDGS. '75-76	X	(?)	X	X	X	X		20/41=49%
TOWN.BLDGS. '77	X	(?)	X	X	X	X		32/51=63%
SUBURBS.BARN '75-76	X	@	X	X	X	X		19/22=86%
SUBURBS.BARN '77	X	@	X	X	X	X		23/36=64%
SUBURBS.HOMES '75-76	X	@	X	X	X	X		25/47=53%
SUBURBS.FIELDS '75-6	X	@	X	X	X	@		0/53
<u>KOKA</u>								
TOWN.BLDGS. '76	X	@	X	X	X	X		3/18=17%
TOWN.BLDGS. '77	X	@	X	X	X	X		0/80
FIELDS. '75-76	X	0/44	X	X	X	0/6	0/50	0/44
FIELDS. '77	X	0/10	X	X	X	@	0/10	0/17
LEMI. '76	0/2*	0/15	X	X	X	X	0/17	0/60
MENAGESHA. '76	@	0/14	X	X	@	X	0/14	0/36
ANKOBER. '75	0/8*	X	0/8*	0/1*	0/10	X	0/27	0/44
<u>MT. INTOTO '77</u>								
K. MEHRET. HOMES	(?)	(?)	X	X	X	X		14/95=15%
MARYAM. HOMES	@	X	X	X	X	X		2/86= 2%
MARYAM. OUTDOORS	0/14	X	@	@	@	X	0/14	0/29
SUBTOTALS								
1975-76	0/10	0/73	0/8	0/1	0/10	0/6	0/108	67/471=14%
1977	0/14	0/10	@	@	@	@	0/24	71/394=18%
TOTALS								
1975-77	0/24	0/83	0/8	0/1	0/10	0/6	0/132	138/865=16%

TABLE 8. RESULTS OF 132 INDIRECT FLUORESCENT TESTS FOR MURINE TYPHUS (R. MOOSERI) INFECTION IN LOPHUROMYS, MASTOMYS AND SOME NON-COMMENSAL RODENTS IN ETHIOPIA, WITH TOTALS FOR ALL 865 TESTS. (1975-1977)

Numerator = Number positive. Denominator = Number tested. X = Rodent not collected and may be absent. @ = Rodent present but not tested. (?) = Not collected, and probably present. \* = Tested with fluorescein-conjugated antiserum to Arvicanthus globulin.



for the period 1975-76 and for 1977. It is clear at a glance that of the 12 species of rodents tested, only 3, Rattus rattus, Mus musculus and Praomys albipes, yielded positive results, and that of the 3, R. rattus was implicated to a far greater degree than the others. Thus, overall, 39% of the 344 samples tested from Rattus were positive, in contrast to 1 of 9 (11%) from Mus musculus, 3 of 165 (2%) from Praomys and 0 of 347 for the other 9 species.

b) In the highlands, Rattus were collected only in the vicinity of Addis Ababa (which includes Mt. Intoto), only indoors, and only at elevations below 2900 m. (i.e. from 2300 m. to 2600 m.). Rattus presumably were absent at Leml and Menagesha and in domiciles at the top of Mt. Intoto. Where found in any of these highland areas, and whenever tested, an impressively high percentage of Rattus were naturally infected with R. mooseri, i.e. from 27% to 86%. The maximum percentages were at the barn at Mekanissa (76% and 86%); the lowest, at Intoto Kedani Mehret. The relatively low rate of infection in Rattus at Koka is noteworthy. In this area, in the Rift Valley, at 1640 m. elevation, all tests for the 88 rats examined were negative, and this includes 80 rats from the buildings in town where 3 of 18 (17%) rats were infected last year. Since the infected rats were from a coffee-shop and hotel along the main road from Addis, the possibility exists that they (or the source of infection) had been imported but discussion of this point is reserved until later (Rept. p. 33; Appl. p. 66). Further details on the rate of infection in Rattus (e.g. distribution by sex and age, etc.) are presented in paragraphs e and f on this page, after consideration of the results with other rodents.

c) Mus musculus and Mus (Leggada) have been inadequately sampled. The former, the house mouse, has been collected only indoors, and of the 9 specimens tested, 1, at Mekanissa, was positive. Last year we reported negative results with 47 Praomys from Menagesha and Leml, and of the 118 samples tested in 1977, all from Intoto, 3 were positive, viz: 1 of 38 (3%) from Intoto Kedani Mehret and 2 of 80 (2.5%) from I. Maryam. Those tested were all from indoors. Of the other rodents, which were all negative, only Arvicanthus (184) and Mastomys (86) were really sampled adequately.

d) It is emphasized that R. mooseri infection in rodents was noted only in areas and habitats where either Rattus or Mus (or both) occurred. This is clear from Table 9, which compares the results of these two categories, and also treats the data whether the hosts were taken in buildings or outdoors. Thus, of the 870 rodents tested in the period 1975-1977, 576 were from hosts collected indoors, and 294 were from field-specimens. Of the 522 rodents sampled from buildings where Rattus and/or Mus musculus were present, 138 (26%) were positive for R. mooseri. Of the 190 Rattus from Addis town and suburbs, 60% were positive. Including the unique Rattus in the Acacia groves at Addis, a total of 208 field-rodents were examined from the areas where Rattus were present, and all were apparently free of R. mooseri infection. There was no evidence of R. mooseri in the 86 indoor murines and 54 outdoor specimens examined from localities where ostensibly there were no Rattus or Mus musculus. Thus, although 2.5% of the Praomys were positive in the first category, all of the 47 examined in the second were deemed negative. The table incorporates data from 1976 at Leml and Menagesha where there were no positive results reported from 71 tests from indoor Mastomys (29), Praomys (36) and Desmomy (6) nor from 13 outdoor specimens of the last two.

(TABLE 9 ABOUT HERE)

e) Table 9 also summarizes data on the Xenopsylla cheopis and X. bantonum and the Leptopsylla associated with these rodents, and shows that regardless whether Rattus or Mus musculus occur in the area or habitat, these Xenopsylla can infest the various murines, either indoors or outdoors. L. segnis, in contrast, is wholly limited to Category I, and there essentially to Rattus and this Mus. L. aethiopica has been taken indoors on hosts like Praomys etc., but not on Rattus, Mus musculus or outdoors on hosts like Arvicanthus.

f) It is noteworthy that there is no significant difference in the rate of R. mooseri infection in female Rattus as compared to males, as shown in Table 10. Regardless of the

	OUT- DOORS	IN- DOORS	NUMBERS	% POSITIVE	X. CHEOPIS	X. BANTORUM	L. SEGNIS	L. AETHIOPICA
I. AREAS WHERE <u>RATTUS</u> AND/OR <u>MUS MUSCULUS</u> ARE PRESENT								
A. <u>RATTUS RATTUS</u>								
1) ADDIS		+	118/190	60%	+	+	+	0
2) KOKA		+	3/105	3%	+	+	0	0
3) KOKA	+		0/1	-	+	+	0	0
4) INTOTO K. MEHRET		+	13/48	27%	+	+	+	0
5) TOTALS FOR <u>RATTUS</u>		+	134/344	39%				
B. <u>MUS MUSCULUS</u>		+	1/9	11%	+	+	+	0
C. <u>MUS (LEGGADA)</u>	+		0/4	-	+	+	0	+
D. <u>PRAOMYS ALBIPES</u>		+	3/118	2.5%	+	+	R	0
E. OTHER NATIVE RODENTS		+	0/52	-	+	+	R	0
F. OTHER NATIVE RODENTS	+		0/198	-	@	@	0	++
G. TOTALS: ALL INDOOR RODENTS SAVE <u>RATTUS</u> AND <u>MUS</u>			3/170	1.8%				
H. TOTALS: ALL INDOOR RODENTS			138/522	26%				
I. TOTALS: ALL OUTDOOR RODENTS			0/208	-				
II. AREAS WHERE <u>RATTUS</u> AND <u>MUS</u> ARE PRESUMABLY ABSENT								
A. <u>PRAOMYS ALBIPES</u>		+	0/47	-	+	+	0	+
B. <u>ARVICANTHIS</u>	+		0/27	-	+	+	0	0
C. OTHER MURINES		+	0/39	-	+	+	0	+
D. OTHER MURINES	+		0/17	-	+	+	0	+
E. <u>TACHYORYCTES</u>	+		0/10	-	0	0	0	0
F. TOTALS FOR OUTDOOR RODENTS	+		0/54	-				
G. TOTALS FOR INDOOR RODENTS		+	0/86					

TABLE 9. SUMMARY OF DATA ON FA TESTS COMPARING RATES IN RATTUS RATTUS AND MUS MUSCULUS WITH OTHER RODENTS (INDOORS AND OUTDOORS) IN ETHIOPIA, WITH INDICATION OF THEIR MAJOR XENOPSYLLA AND LEPTOPSYLLA FLEAS (1975-1977)

Numerator = Number positive. Denominator = Number tested.

+ = Present. 0 = Absent. @ = Does not apply to Tachyoryctes.

R = Rarely. \* = Only on native "mice", etc.

over-all rate of infection in an area, the percentage of infected males was always essentially the same as in females, indicating the observation is valid.

LOCALITY	MALES		FEMALES	
	NUMBERS	% POSITIVE	NUMBERS	% POSITIVE
ADDIS ABABA Town	25/40	63%	27/45	60%
ADDIS SUBURBS (MAKANISSA) Village	7/11	64%	8/12	67%
Barn	16/20	80%	21/27	78%
ADDIS - MT. INTOTO Intoto Kedani Mehret	4/17	26%	8/31	26%

TABLE 10. MURINE TYPHUS INFECTION RATES BY SEX AND LOCALITIES AMONG RATTUS RATTUS COLLECTED IN ETHIOPIA (1976-1977)

Numerator = Number positive. Denominator = Number tested.

g) When age is considered, however, the results are strikingly different, being much higher in adults than in juveniles. As shown in Table 11, this difference ranged from 6% to 45% higher in adults. These data suggest that while R. mooseri infection may be acquired early in life, the factor of time, i.e. duration of exposure, is important. Presumably older rats have had more opportunity to come into contact with the agent.

LOCALITY	JUVENILES		ADULTS	
	NUMBERS	% POSITIVE	NUMBERS	% POSITIVE
ADDIS ABABA Town	8/15	53%	44/69	64%
ADDIS SUBURBS (MAKANISSA) Houses	4/7	57%	11/16	69%
Barn	6/13	46%	31/34	91%
ADDIS - MT. INTOTO Intoto Kedani Mehret Houses	2/10	20%	10/38	26%
TOTALS	20/45	44%	96/157	61%

TABLE 11. MURINE TYPHUS INFECTION RATES AMONG ETHIOPIAN RATTUS RATTUS BY AGE. (VICINITY OF ADDIS ABABA) (1976-1977)



h) In most instances samples of sera were obtained from the Rattus which were used for collection of blood on filter paper. The titers for the R. rattus sera which proved positive to the IFA test for R. mooseri in 1976-77 are shown in Table 12, whence it can be seen that in 87 of the 119 positive tested (73%) the titers were 1:160 or greater, and in 40% (48 instances) the titers were 1:640 or greater.

LOCALITY	NO. POSITIVE/ NO. EXAMINED@	TITER*							
		1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
ADDIS TOWN Houses									
	1976 20/33	3	5	-	10	-	1	1**	-
	1977 32/51	2	3	3	2	12	5	2	3
	SUBTOTAL 52/84	5	8	3	12	12	6	3	3
ADDIS SUBURBS (MAKANISSA) Houses									
	(1976) 15/23	2	3	-	7	-	3	-	-
	Barn								
	1976 15/18	1	3	0	10	0	1	0	-
	1977 22/29	2	1	1	2	11	4	1	-
	SUBTOTAL 37/47	3	4	1	12	11	5	1	-
ADDIS. MT. INTOTO Intoto Kedani Mehret									
	(1977) 12/48	3	2	3	-	2	2	-	-
KOKA (Town and Village)									
	1976 3/26	1	1	-	1	-	-	-	-
	1977 0/80	-	-	-	-	-	-	-	-
	SUBTOTAL 3/106	1	1	-	1	-	-	-	-
TOTALS	119/308	13	19	7	32	25	16	4	3

TABLE 12. TITERS OF RATTUS RATTUS SERA POSITIVE TO THE INDIRECT FLUORESCENT ANTIBODY TEST FOR MURINE TYPHUS (1976-1977).

@ = Sera was not available for every rat from which blood on filter paper was obtained. \* = End-point only. \*\* Male juvenile.

Additional Notes. Mus musculus from Makanissa Barn with titer 1:640. Praomys albipes from Intoto Kedani Mehret 1:80. Praomys albipes from Intoto Maryam 1:80, 1:40.

IFA Test performed only for presence of R. mooseri.

1) It should be noted that in the areas where the largest proportions of Rattus harbored R. mooseri (Addis Town and Makanissa), the titers in general were significantly higher than in areas like Koka and Intoto Kedani Mehret, as shown in Table 13. This may be of epidemiological significance, but there are no data to show that where there is a low rate of natural infection of R. mooseri, the strains may be less virulent. Such a phenomenon has been noted for chigger-borne rickettsiosis (Traub & Wisseman, 1974), however, and in this regard there is a pertinent parallel with that rickettsiosis, namely, that these localities may be at the periphery of the ecological (or local geographical) range of the infection. Here the limits may be the temperature and rainfall affecting Rattus and Xenopsylla (and other fleas?) on upper Mt. Intoto, and at Koka, the effect of the heat and aridity on Rattus.

AREA	TITERS WITH 1:320 OR GREATER	
	NUMBERS	PERCENT
ADDIS ABABA. TOWN	36/52	70%
ADDIS SUBURBS		
A. BARN	29/37	78%
B. HOUSES	10/15	67%
INTOTO KEDANI MEHRET	4/12	33%
KOKA	1/3	33%

TABLE 13. NUMBERS OF RATS POSITIVE FOR R. MOOSERI  
 WITH TITERS OF 1:320 OR GREATER

f. Direct Fluorescent Antibody Tests (FA) with Fleas

1) The data for both 1976 and 1977 are presented in Table 14, whence again it can readily be seen that almost all the positive fleas came from Rattus, and that they were restricted to the Addis area (including Intoto). The demonstration of R. mooseri in fleas in 1977 was at a lower rate than last year, e.g. 8% in Addis town versus 43% last year, and a reduction in the percentage of infected L. segnis from 46% to 10% in that area. Possible reasons for this phenomenon are discussed below (p. 34). Once again the highest rates of infection were observed in L. segnis (e.g. 9% at Mekanissa) versus a maximum of 3.3% in X. bantorum, also from that area. In fact, no other infected Xenopsylla of either X. cheopis or X. bantorum were noted this year. For the first time infected fleas were observed in an area outside of Addis town or its suburb at Mekanissa, i.e. at Intoto K. Mehret, which is just 2 km. from Addis proper. Here 8 of 178 (4.5%) L. segnis from Rattus were positive. The absence of demonstrable infection in fleas at Koka is noteworthy, especially since 100 Xenopsylla and 70 Echidnophaga n.sp., all from Rattus in domiciles, were examined. It will be recalled that all the Rattus fleas were likewise negative this year.

(TABLE 14 ABOUT HERE)

2) The data on the FA test with fleas are summarized in Table 15, facilitating analysis. Out of the total of 1224 fleas of all kinds dissected and tested in 1976-77, 873 were from Rattus, and 80 (10%) of these were positive. There were 245 from Praomys and 106 from all other hosts, and these were all negative. Regarding Rattus from the Addis area (excluding Intoto), only 3 of 59 X. cheopis (5%) and 8 of 81 (10%) of the X. bantorum had demonstrable R. mooseri, whereas 19% of 323 L. segnis were infected. Of the 210 Rattus fleas examined from Intoto, 8 (4%) were positive, but these were from the 178 L. segnis tested. None of the 245 Praomys fleas from Intoto and other areas were infected although R. mooseri infection in some of those hosts had been noted in Intoto. Altogether, 3 of 181 X. cheopis (2%) and 16 of 268 (6%) X. bantorum were positive, and these were all from Addis town or Mekanissa (and were from Rattus). Of the 511 L. segnis checked, 69 (13%) were positive, and all but 8 of those came from Addis town or Mekanissa. The remainder were from Intoto. The low rate in Xenopsylla as compared to L. segnis is noteworthy. The highest rate of infection was observed in Addis town, where 9% of the X. cheopis, 15% of the X. bantorum and 26% of the L. segnis had R. mooseri. These high rates no doubt reflect the hyperendemicity in the minifocus mentioned below (p. 24). It is of interest that no infected L. aethiopica were found, and it is stressed again that this species had not been found on Rattus. All the infected fleas to date have been from Rattus.

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 ENTITLED "CLINICAL AND EPIDEMIOLOGICAL STUDIES ON RICKETTSIAL INFECTIONS" -  
 APPLICATION FOR RENEWAL FOR YEAR-5 (1977-1978)

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HOST AND LOCALITY	XENOPSYLLA		LEPTOPSYLLA		ECHIDNO- PHAGA N.SP.	OTHER	TOTALS
	CHEOPIS	BANTORUM	SEGNIS	AETHIOPICA			
1. <u>RATTUS</u>							
ADDIS. TOWN							
'76	3/9 =33% (5)	6/17 =33% (7)	32/70 =46% (13)	-	-	-	41/96 =43%
'77	0/24 = (12)	1/30 =3.3% (2)	9/86 =10% (8)	-	-	-	10/140 = 8%
ADDIS. MAKANISSA							
(BARN)							
'76	0/12 (8)	1/15 = 7% (12)	9/50 =18% (8)	-	-	-	10/77 =13%
'77	0/14 (12)	0/19 (12)	11/117 = 9% (13)	-	-	-	11/150 = 7%
INTOTO KEDANI MEHRET							
'76	-	-	-	-	-	-	-
'77	0/14 (5)	0/18 (5)	8/178 =4.5% (21)	-	-	-	8/210 = 4%
KOKA. TOWN	'76 0/4 (2)	0/6 (2)	-	-	0/20 (2)	-	0/30
KOKA. DOMICILES	'77 0/45 (10)	0/55 (10)	-	-	0/70 (7)	-	0/170
2. <u>PRAOMYS</u>							
LEMI + MENAGESHA	'76 0/9 (9)	0/41 (9)	-	0/20 (5)	-	-	0/70
INTOTO KEDANI MEHRET	'77 0/12 (5)	0/17 (5)	0/10 (2)	0/88 (12)	-	CTENOPH. 0/6 (3)	0/133
INTOTO MARYAM	'77 0/7 (2)	0/11 (2)	-	0/24 (6)	-	-	0/42
3. <u>MASTOMYS</u>							
LEMI + MENAGESHA	'76 0/1 (1)	0/9 (2)	-	-	-	-	0/10
KOKA. FIELDS	'76 0/1 (2)	0/9 (1)	-	-	-	-	0/10
KOKA. HUT	'77 -	-	-	-	-	DIKOPSYL. 0/3 (1)	0/3
ADDIS. MAKANISSA	'76 -	-	-	-	-	DIKOPSYL. 0/10 (4)	0/10
4. <u>ARVICANTHIS</u>							
KOKA. HUTS	'77 0/14 (2)	0/6 (2)	-	-	-	-	0/20
5. <u>CROCIDURA</u>							
KOKA. HUT	'77 0/11 (2)	0/9 (2)	-	-	-	-	0/20
LEMI. HOUSE	'76 0/4 (1)	0/5 (1)	-	-	-	-	0/9
6. <u>ICTONYX</u>							
KOKA. HOUSE	'77 -	-	-	-	0/10 (1)	-	0/10
7. <u>LEPUS</u>							
KOKA. FIELD	'77 -	-	-	-	-	C.FELIS 0/10 (1)	0/10
8. <u>DESMOMYS</u>							
LEMI. HOUSE	'76 -	0/1 (1)	-	-	-	-	0/1
9. <u>HOMO</u>							
MAKANISSA	'76 -	-	-	-	-	P. IRRITANS 0/3 (2)	0/3
TOTALS	'76 3/40 = 8%	7/103 = 7%	41/120 = 34%	0/20	0/20	0/13	51/316 = 16%
'77	0/141	1/165 = 1%	28/391 = 7%	0/112	0/80	0/19	29/908 = 3%
GRAND TOTALS	3/181 =27%	8/268 = 3%	69/511 = 13%	0/132	0/100	0/32	80/1224 = 7%

TABLE 14. NUMBERS OF FLEAS FOUND POSITIVE FOR R. MOOSERI INFECTION IN ETHIOPIA BY DIRECT FA TEST (1976-1977)

Numerator = Number positive. Denominator = Number tested. ( ) = Number of hosts providing fleas for testing. Blank = Not applicable.



	XENOPSYLLA		LEPTOPSYLLA		ALL OTHERS	TOTALS
	CHEOPIS	BANTORUM	SEGNIS	AETHIOPICA		
<u>RATTUS</u>						
ADDIS. TOWN	3/33	7/47	41/156	-	-	51/236
	9%	15%	26%	-	-	22%
MAKANISSA	0/26	1/34	20/167	-	-	21/227
		3%	12%	-	-	9%
ADDIS. TOTALS	3/59	8/81	61/323	-	-	72/463
	5%	10%	19%	-	-	16%
INTOTO KEDANI MEHRET	0/14	0/18	8/178	-	-	8/210
			4%	-	-	4%
KOKA. TOTALS	0/49	0/61	-	-	0/90	0/200
TOTAL FOR RATTUS	3/122	16/160	69/501	-	0/90	80/873
	2%	10%	14%	-		10%
PRAOMYS. ALL AREAS	0/28	0/69	0/10	0/132	0/6	0/245
ALL OTHER HOSTS	0/31	0/39	-	-	0/26	0/106
TOTALS FOR ADDIS	3/59	8/81	61/323	-	0/3	72/466
ALL HOSTS	5%	10%	19%	-		16%
TOTALS FOR KOKA	0/78	0/79	-	-	0/123	0/280
ALL HOSTS						
TOTALS FOR INTOTO	0/21	0/28	0/10	0/112	0/6	0/177
TOTALS -	3/181	16/268	69/511	0.132	0/132	80/1224
ALL HOSTS AND AREAS	2%	6%	13%			7%

TABLE 15. SUMMARY: NUMBERS OF FLEAS FOUND POSITIVE FOR R. MOOSERI INFECTION IN ETHIOPIA BY DIRECT FA TEST (1976-1977)

Numerator = Number positive. Denominator = Number tested.

3) There was no significant difference between the rate of infection noted in male fleas and females. All the positive fleas were associated with the indoors as in the past. No evidence of R. mooseri was found in fleas (or hosts) collected outside buildings, although only limited numbers of such fleas have been examined because our intensive studies have failed to show any R. mooseri infection in outdoor rodents, and hence we concentrated on the most likely vectors in order to get quantitative data rather than dilute our efforts.

4) Hyperendemic Foci of Murine Typhus

a) Last year we presented data strongly indicating the occurrence of hyperendemic foci in highly localized areas of Addis Ababa (town and suburbs). The observations this year support the hypothesis that the infection rate in rats and ectoparasites in certain domiciles or buildings (e.g. the barn at Makanlssa) is significantly higher than in other locations in the general areas, as suggested by the data in Tables 16 and 17. The figures for both 1976 and 1977 are presented in these Tables, which deal with the infection rate in Rattus and lice and/or fleas when at least one ectoparasite was found positive by FA tests. From these it can be seen that in IFA tests with hosts harboring at least one positive louse or flea, 26, or 84%,

RATTUS RATTUS			FLEAS				LICE		
ACCESS. NO.	INFECTED	SOURCE	XENOPSYLLA		LEPTO. SEGNIS	SUBTOTAL	POLYPLAX SPINULOSA	HOPLO- PLEURA	SUBTOTAL
			BANTORUM	CHEOPIS					
B-93013	3+	MAKAN. BARN			1/10 = 10%	1/10 = 10%	0/7		0/7
B-93019	2+	AMHARA. HOUSE			1/3 = 33%	1/3 = 33%	0/8	0/2	0/10
B-93028	3+	AMHARA. HOUSE			1/3 = 33%	1/3 = 33%	1/5 = 20%		1/5 = 20%
B-93043	2+	AMHARA. HOUSE	1/3 = 33%		4/6 = 67%	5/9 = 55%	0/4		0/4
B-93047	2+	ADDIS. HOUSE			1/8 = 13%	1/8 = 13%	0/9	0/1	0/10
B-88724	POS.	MAKAN. HOUSE	0/1		2/8 = 25%	2/9 = 22%			
B-88736	NEG.	MAKAN. BARN			0/10	0/10	1/2 = 50%	0/2	0/4 = 25%
B-88750	POS.	AMHARA. HOUSE	2/2 = 100%	1/2 = 50%	2/4 = 50%	5/8 = 63%	0/1	0/1	0/2
B-88751	NEG.	AMHARA. HOUSE	4/4 = 100%	1/1 = 100%	5/5 = 100%	10/10 = 100%			
B-88752	NEG.	AMHARA. HOUSE		1/1 = 100%	1/1 = 100%	2/2 = 100%			
B-88773	POS.	MAKAN. BARN			2/5 = 40%	2/5 = 40%		0/1	0/1
B-88792	POS.	AMHARA. HOUSE	1/5 = 20%	0/5		1/10 = 10%		1/1 = 100%	1/1 = 100%
TOTALS	9/12 = 75%		8/15 = 53%	3/9 = 33%	20/63 = 32%	31/87 = 34%	2/36 = 6%	1/8 = 13%	3/44 = 7%

TABLE 16. RESULTS OF IFA TESTS IN WHICH AT LEAST ONE FLEA OR LOUSE WAS POSITIVE FOR R. MOOSERI AND WHERE ANOTHER SPECIES OF ECTOPARASITE WAS ALSO TESTED

Numerator = Number positive. Denominator = Number tested. POS. = Positive for R. mooseri. NEG. = Negative. 1+ to 3+ = Indication of positive titer (not available for 1976). B-93013 et seq. = 1977, remainder = 1976.

of the Rattus were positive, whereas our data show that the percentage for the other Rattus in the same areas (Addis town, Makanissa suburb and Intoto) was 49%. Specifically, the rates were: Addis town 88% versus 55%; Makanissa 84% vs. 72% and Intoto 50% vs. 2%. Thus, if one infected flea or louse was present on the Rattus, the chances of the rat having been infected with R. mooseri seem far greater than if the fleas examined had been negative. However, it might be argued that this is to be expected, in that if the Rattus were positive, then it is quite likely that fleas or lice feeding on it could acquire the rickettsiae, and that is what these figures suggest. Analysis tends to refute that contention. Rats are infected for life, but rickettsemia is transient and probably only persists for a few days. This alone indicates that recently acquired infection in a single rat could not result in such a high correlation as noted above. Moreover, 19 Rattus from Addis town and suburbs, which were positive by IFA, carried a total of 39 Xenopsylla and 88 L. segnis that were negative by FA test. Also, there were 12 positive Rattus from Intoto which had negative fleas, i.e. there were 190 negative Intoto L. segnis and Xenopsylla. Therefore, since so many positive rats carried fleas lacking R. mooseri, it seems logical that other factors are involved besides infected rats merely carrying positive fleas by virtue of concurrent rickettsemia. Further, as shown in Table 16, when one species of flea was found positive on the host, other species of fleas (if present) also were highly likely to be infected, and the same is apparently true for the Rattus lice. Thus, under those circumstances, in 6 of 12 cases (50%), at least one other species was positive as well, and in 13 of the 23 groups containing at least 1 infected individual more than 39% of the individuals of that species harbored R. mooseri. In 8 instances, all the members of that species were infected per host. Once, every member tested for 3 species of fleas from an individual rat was positive, and in another example, 100% infection was noted for one species of flea and 2 of lice from a single Rattus. There was one other example of infection in all 3 species of fleas on one host. In these cases of multiple infestation where one species

RATTUS RATTUS			FLEAS			LICE		
ACCESS. NO.	INFECTED	SOURCE	XENO- PSYLLA	LEPTO. SEGNIS	SUBTOTAL	POLYPLAX SPINULOSA	HOPLO- PLEURA	SUBTOTAL
B-93001	3+	MAKANISSA. BARN		6/10= 60%	6/10= 60%			
B-93018	3+	AMHARA		1/3 = 33%	1/3 = 33%			
B-93038	2+	MAKANISSA. BARN		1/10= 10%	1/10= 10%			
B-93044	2+	AMHARA		1/1 =100%	1/1 =100%			
B-93045	3+	AMHARA		4/4 =100%	4/4 =100%			
B-93049	3+	MAKANISSA. BARN		2/5 = 40%	2/5 = 40%			
B-93050	NEG.	MAKANISSA. BARN		1/5 = 20%	1/5 = 20%			
B-93271	3+	INTOTO		7/10= 70%	7/10= 70%			
B-93409	NEG.	INTOTO		1/2 = 50%	1/2 = 50%			
B-88738	POS.	AMHARA		7/9 = 78%	7/9 = 78%			
B-88739	POS.	AMHARA		10/10=100%	10/10=100%			
B-88769	POS.	MAKANISSA. BARN		5/10= 50%	5/10= 50%			
B-88779	POS.	AMHARA				1/1 =100%		1/1=100%
B-88781	POS.	MAKANISSA.HOUSE					1/1=100%	1/1=100%
B-88782	POS.	MAKANISSA.HOUSE					1/1=100%	1/1=100%
B-88787	POS.	MAKANISSA.HOUSE				1/1 =100%		1/1=100%
B-88791	POS.	AMHARA		6/10= 60%	6/10= 60%			
TOTALS	16/18=90%			52/89= 57%	52/89= 57%	2/2 =100%	2/2=100%	4/4=100%

TABLE 17. RESULTS OF IFA TESTS IN RATTUS AND OF DIRECT FA TESTS IN RAT FLEAS AND LICE IN WHICH ONLY ONE SPECIES OF ECTOPARASITE WAS TESTED AND IN WHICH AT LEAST ONE SPECIMEN WAS POSITIVE FOR R. MOOSERI INFECTION

Numerator = Number positive. Denominator = Number tested. POS. = Positive for R. mooseri. NEG. = Negative. 1+ to 3+ = Indication of positive titer (not available for 1976). B-93001 et seq. = 1977, remainder = 1976.

was infected, the over-all percentage of fleas or lice that had R. mooseri ranged from 6% to 53%. Nine of these 12 host Rattus (75%) were naturally infected with R. mooseri.

b) The high rate of infection noted in ectoparasites when one member of a group from a Rattus was found to be infected, is apparent from the data in Table 17. There were 89 L. segnis tested from 13 Rattus which had infected members of that species, and 57% of the fleas examined had R. mooseri. There was a high rate of infection in the rat lice, viz. 4 individuals of 4 tested from 4 Rattus in 1976 were positive, but the sampling is inadequate for further discussion. Sixteen of the 18 Rattus (90%) providing these infected ectoparasites were themselves positive in IFA tests.

c) These data on R. mooseri in Rattus-ectoparasites suggest hyperendemicity in certain foci or at certain times. When an individual louse or flea became infected, a significant proportion of other lice and/or fleas on the same host were positive, indicating either a recent pervasive rickettsemia and/or an abundance of infected hosts, one of which had previously infected those ectoparasites. The great majority of rats (25 of 30, or 80%) with infected lice or fleas were themselves positive for R. mooseri. Moreover, the bulk of the infected Rattus and ectoparasites came from two small foci, namely Amhara's house in Addis, and the dairy barn in Makanissa. Thus, of the 30 rats represented in



Tables 16 and 17, 26 (87%) came from either of these 2 places (14 from Amhara's house, and with 12 (86%) infected, and 12 from the barn, with 10 (83%) infected.) Further, altogether (including Rattus with negative fleas or lice), a total of 23 Rattus were collected in Amhara's house in 1977, and 18 (78%) were infected. Last year the rate in that domicile was  $7/9 = 77\%$ , whereas the rate for other parts of Addis was 58%. An important point is that in 1977 a period was reached when it was impossible to trap rats in Amhara's house for weeks, indicating that either no Rattus remained or they had become too wily to trap. After a lapse of 3 weeks an additional 4 rats were taken, and these were all negative. All in all these observations suggest that these last 4 were recent arrivals that had not yet become demonstrably infected with R. mooseri. In the barn in the suburbs of Addis in 1976, 94% of the adult Rattus were positive for R. mooseri by IFA. The rates for all Rattus there in 1976 and 1977 respectively were 86% and 77%. Insofar as infected ectoparasites in these particular foci are concerned, there were 30 instances in which one or more such fleas or lice were encountered and 24 (80%) of these concerned either the barn or Amhara's house. Of the 12 instances of multiple occurrence of infected ectoparasites of two or more species, 11 (91%) were from one of these two localities. We are referring to such highly localized areas of hyperendemicity as minifoci (vide p. 28, on the summary of epidemiological considerations).

g. Direct FA Tests on Lice

1) Lice on Rattus in Ethiopia have proven difficult to collect, and for that reason, and because of severe constraints of time we have not been able to prepare optimal numbers of dissections of Rattus-lice, particularly in the past. This year we tested lice from 10 Rattus from the Addis area, from 7 from Koka and from 6 from Intoto, as well as from 5 Praomys and 1 Lophuromys from Intoto. Lice from 2 Arvicanthis at Koka were also checked.

2) There are two species of lice occurring together on Rattus in Addis (and other areas of Ethiopia), namely Polyplax spinulosa and Hoplopleura oenomydis, both of which are widely distributed with these rats in various parts of the world.

3) Polyplax outnumbered Hoplopleura this year in our samples by about 3 to 1. Identifications on the species on Praomys and Arvicanthis are pending. The numbers of lice tested from these various hosts are shown in Table 18.

	RATTUS	PRAOMYS	ARVICANTHIS	LOPHUROMYS (L) OR MASTOMYS (M)
ADDIS. TOWN	53 (6)	-	-	-
ADDIS. MAKANISSA (BARN)	38 (4)	-	-	-
INTOTO	45 (5)	44 (5)	-	5 (1=L)
KOKA	64 (7)	-	20 (2)	8 (1=M)
TOTALS	200 (22)	44 (5)	20 (2)	13 (2)

TABLE 18. NUMBERS OF LICE FROM DESIGNATED HOSTS AND AREAS IN ETHIOPIA EXAMINED BY DIRECT FA TEST (1977)

Figures in parentheses indicate the numbers of hosts supplying these lice.

4) The results with the lice in 1976 and 1977 are summarized in Tables 16 and 17, and some of the pertinent points have been discussed immediately above. Last year we examined 18 lice from 13 Rattus, and 6 of these lice were positive (3 of each species). Eleven of those rats had contributed 1 louse apiece. Neither in that year nor in the current one were any infected lice found on any host besides Rattus. In 1977, only 1 louse (P. spinulosa) was positive in our tests, and it came from a Rattus in Amhara's house, and its 4 companions were negative (but 1 of 3 L. segnis from that host was likewise positive).

h. Direct FA Tests on Ticks and Mesostigmatid Mites

1) Last year we reported that a total of 78 ticks, representing various stages in 4 genera, were all negative for murine typhus. Similar results were obtained in 1977 with 195 unidentified ticks, as shown in Table 19 (which also deals with mesostigmatid mites and the spotted fever group of rickettsiae, as discussed below). The results with ticks and R. mooseri were also negative in 1975. However, it must be borne in mind that very few ticks from Rattus have been examined, and that host is the prime theraphion found infected to date. There have been no ticks found on Rattus in Addis Ababa and its suburbs, where the bulk of the infected Rattus have been taken. The few Rattus ticks examined have been from Koka, where the R. mooseri rate is very low in Rattus (and unreported in other hosts), and the ticks have come from Rattus in huts in the fields and not in town, which was the source of the few positive Rattus observed.

2) As for the mesostigmatid mites, the situation re murine typhus resembles that noted in ticks - the results to date are all negative, including the 140 tested in 1977 (Table 19). However, very few Rattus mites have been checked, although in 1977 we tested one pool from a host at Makanissa that was positive by IFA.

(TABLE 19 ABOUT HERE)

i. Isolation of R. mooseri from Field-Collected Rodent Tissues

1) A truly signal advance was made in 1976 when isolations were made directly into tissue cultures from kidney tissues collected from Rattus in Ethiopia, derived from hosts that had proven positive for R. mooseri by the IFA test. The successful development of this technique was a crucial step in our program, because it became possible to accurately and relatively quickly identify and characterize strains of R. mooseri encountered in the field, and the methodology should also prove applicable to identification of other species of rickettsiae that may be encountered in the field, particularly in native murines. This new technique eliminates the problem of enzootic rickettsiae and other microorganisms of guinea pigs, etc. which have plagued research when laboratory animals were used for isolation of agents. It is hoped that this technique may prove feasible for isolation of rickettsiae from arthropods, but that will be a difficult and time-consuming task to accomplish. Data on the strains in tissue culture are presented in Table 20.

(TABLE 20 ABOUT HERE)

2) The rickettsiae isolated to date are considered to be typical R. mooseri.

j. Summary and Discussion of Observations on the Ecology of Murine Typhus.

1) In order to facilitate discussion, the highlights of the data and observations are summarized in Tables 21 and 22. From the points summarized therein and/or made earlier,

(TABLES 21 AND 22 ABOUT HERE)

it is clear that murine typhus infection in the areas studied in Ethiopia is characterized by the following: 1) The invariable presence of commensal Rattus rattus or at least commensal Mus musculus. 2) Indoor foci. 3) An occasional occurrence in native murines, but only when indoors and acting like a commensal rodent and then only in association with R. rattus or M. musculus. 4) Where R. mooseri is common in Rattus, the flea Leptopsylla segnis is abundant on such hosts and has a relatively high rate of infection. The only area known to us where R. mooseri is present in Rattus in the absence of L. segnis is Koka town, and here the rate of infection in those rats is very low. 5) Presence of both X. cheopis and X. bantonum indoors on

LOCALITY AND ACCESSION NUMBERS	HOST	TICKS				MITES			
		MURINE TYPHUS		SPOTTED FEVER		MURINE TYPHUS		SPOTTED FEVER	
		#	%	#	%	#	%	#	%
1. <u>ADDIS. TOWN. HOUSES</u> AZ 936B	RATTUS					0/10		0/10	
2. <u>ADDIS. MAKANISSA. BARN</u> AZ 926 AZ 950 AZ 958 AZ 966	HORSES HORSES CATTLE CATTLE	0/10 0/10 0/10 0/10		0/10 0/10 0/10 0/10					
3. <u>INTOTO KEDANI MEHRET</u> AZ 963 AZ 1365 AZ 1612 AZ 1191 AZ 1555 AZ 1194	PRAOMYS PRAOMYS PRAOMYS DESMOMYS DESMOMYS DESMOMYS					0/10 0/10 0/10 0/10 0/10 0/10		0/10 0/10 0/10 0/10 4/10	
4. <u>INTOTO MARYAM</u> AZ 987 AZ 1012 AZ 1045	PRAOMYS PRAOMYS PRAOMYS	0/10		0/10				0/10 0/10 0/10	
5. <u>KOKA. TOWN. HOUSES</u> AZ 1056 AZ 1062 AZ 1119 AZ 1158	RATTUS RATTUS RATTUS RATTUS					0/10 0/10 0/10 0/10		0/10 0/10 0/10 0/10	
6. <u>KOKA. HUTS. IN FIELD</u> AZ 1073 AZ 1087 AZ 1099 AZ 1117 AZ 1116 AZ 1122	ICTONYX ARVICANTHIS ARVICANTHIS ARVICANTHIS RATTUS GENETTA	0/15 0/10 0/20 0/10 0/10 0/20		0/15 0/10 0/20 0/10 0/10 0/20		0/10 0/10		0/10 0/10	
7. <u>KOKA. IN ACACIA</u> AZ 1074 AZ 1095 AZ 1166 AZ 1168	LEPUS MASTOMYS HERPESTES CATTLE	0/10 0/10 0/10 0/10		0/10 0/10 1/10 0/10	10%				
TOTALS		0/195		1/195	0.5%	0/140		4/140	3%

TABLE 19. RESULTS OF DIRECT FA TESTS WITH TICKS AND MESOSTIGMATID MITES REGARDING MURINE TYPHUS AND THE SPOTTED FEVER GROUP OF INFECTIONS IN ETHIOPIA (1977)  
 Numerator = Number positive. Denominator = Number tested.



RAT ACCESS. NO.	LOCALITY AND HABITAT	PFU/GR. OF TISSUE (WET WEIGHT)		SERA TESTED FOR MT ANTIBODIES	
		SPLEEN	KIDNEY	IFA	MA
AZ 333	ADDIS. TOWN	$4.12 \times 10^2$	$3.15 \times 10^4$	2560	256
AZ 357	ADDIS. BARN	0	$6.65 \times 10^1$	1280	128
AZ 306	ADDIS. TOWN	$1.35 \times 10^5$	$7.20 \times 10^4$	320	128
AZ 331	ADDIS. TOWN	$2.0 \times 10^4$	$6.40 \times 10^3$	1280	64
AZ 305	ADDIS. TOWN	0	$4.10 \times 10^3$	320	256
AZ 319	ADDIS. SUBURB	0	$2.50 \times 10^3$	80	256
AZ 325	ADDIS. BARN	$7.35 \times 10^3$	$1.37 \times 10^5$	320	320
AZ 332	ADDIS. TOWN	$1.87 \times 10^5$	$2.5 \times 10^5$	320	320

TABLE 20. DETERMINATION OF MURINE TYPHUS RICKETTSIAL  
 PLAQUE-FORMING UNITS IN VARIOUS ETHIOPIAN  
 RAT TISSUES

Rattus or Mus musculus. 6) Infestation of Polyplax spinulosa and Hoplopleura oneomydis on Rattus. 7) X. cheopis and X. bantorum on native murines in the field seem to play no role, even though they may be 20-50 times as abundant as those Xenopsylla on those hosts or Rattus indoors in the same area. 8) The same is true for native species of fleas whether on indigenous rodents or on Rattus in houses (as Echidnophaga n.sp. in Koka). 9) Indigenous rodents in the field do not seem to acquire natural infection, even when living a few yards from a mini-focus like the barn at Makanissa. 10) There is no evidence that parasitic mites and ticks are involved in the cycles.

2) Except for the presence of R. mooseri, which of course is axiomatic, there is no single factor which is invariably associated with, or limited to, endemic foci of murine typhus infection in Ethiopia. Even Rattus is not inevitably present, as seems to be the case at Intoto Maryam, which may be too cold for that host, and where Mus musculus may have usurped its role. However, it is quite likely that an occasional infected Rattus may be introduced into a hut there by the agency of man and survive. While X. cheopis and X. bantorum have been found wherever R. mooseri was demonstrated in these study-areas, they are uncommon there, and moreover, may be extremely abundant on their true hosts (Arvicanthis and Mastomys) (outdoors) in such localities in the absence of infection in those hosts or in the fleas themselves. L. segnis had the highest rate of R. mooseri infection of all the ectoparasites tested in adequate numbers, e.g. 46% of those sampled were positive in Addis town in 1976, but its absence in Koka shows that it cannot be the critical factor in determining the distribution of the rickettsiosis. The rat-lice Polyplax and Hoplopleura seem to be found on Rattus in all areas tested, and a high rate of infection has been noted, e.g. 1 of 1 positive on 4 occasions, but of 200 Rattus-lice tested in 1977, only 1 was found infected. These new data militate against these lice as being the dominant vector in the ecology of murine typhus, even among rats, but may represent inadequate sampling. The role of Hoplopleura and Polyplax needs further study. Regardless, there

	RATTUS RATTUS IN BLDGS. FIELDS BLDGS.	MUS MUSCULUS IN BLDGS. FIELDS BLDGS.	ARVICANTHIS IN BLDGS. FIELDS BLDGS.	MASTOMYS IN BLDGS. FIELDS BLDGS.	PRIONYX IN BLDGS. FIELDS BLDGS.	DESMONYX IN BLDGS. FIELDS BLDGS.	X. CHEOPIS and X. BANTORUM IN BLDGS. FIELDS BLDGS.	L. SEGNIIS IN BLDGS. FIELDS BLDGS.
ADDIS TOWN	+ N.A.	@ N.A.	X N.A.	(?) N.A.	X N.A.	X N.A.	+ N.A.	+ N.A.
ADDIS SUEURBS	+ X	+ X	X 0	@ @	X X	X X	+ Very Rare	+ X
KOKA TOWN	+ N.A.	(?) N.A.	X N.A.	@ N.A.	X N.A.	X N.A.	0# N.A.	X X
KOKA FIELDS & GROVES	0# Very Rare	(?) X	0# 0	0# 0	X X	X X	0# 0	X X
LEMI	X X	@ X	X 0#	0# 0#	0* 0#	0#* 0#	0# 0#	X X
MEMAGESHA	X X	@ X	X X	0# 0#	0#* 0#	0#* 0#	0# 0#	X X
ANKOBER	X X	(?) X	(?) 0	X X	X X	X X	X X	X X
INTOTO KEDANI MEHRET	+ N.A.	0# N.A.	X @	(?) N.A.	+ 0	0# 0#	0 N.A.	+ N.A.
INTOTO MARYAM	X X	0# X	@ 0	X X	+ @	0# 0#	0 0	Rare@ X

TABLE 21. SUMMARY OF DATA ON R. MOOSERI INFECTION IN CERTAIN RODENTS (IFA TEST, 1975-77) AND IN X. CHEOPIS,  
X. BANTORUM AND L. SEGNIIS FLEAS (DIRECT FA TEST, 1976-77) IN BUILDINGS AND FIELDS IN ETHIOPIA

+ = Some samples found positive. N.A. = Not applicable. 0 = Samples tested were negative.  
@ = Species present, but not tested. # = Limited sampling. X = Not collected and may be  
absent. (?) = May occur, but not collected. \* = Tested with fluorescein-conjugated anti-  
sera to Mastomys or Arvicanthhis globulin.

	FROM RATTUS RATTUS				FROM OTHER HOSTS						MITES	TICKS
	X. CHEOPIS	X. BANTORUM	L. SEGNIS	LICE	MITES	X. CHEOPIS	X. BANTORUM	L. AETHIOPICA	DINOP. & CTINOPH- THALINUS N. SP.	PHAGA		
ADDIS TOWN	+	+	+	+	O#	X	X	X	X	X	@	X
ADDIS SUBURBS	+	+	+	+	@	X	X	@	O#	X	O	O#
KOKA TOWN	O#	O#	X	O#	O#	@	@	X	O#	O#	@	X
KOKA FIELDS & GROVES	O#	O#	X	O#	@	O	O	@	X (CT) O# (DI)	@	O	O
LEMI	O	O	X	X	X	O	O	O	@	X	@	O#
MENAGESHA	O#	O#	X	X	X	O#	O#	O#	@	X	@	@
ANKOBER	X	X	X	X	X	X	X	X	@	X	@	@
INTOTO KEDANI MEHRET	O	O	+	O#	@	O#	O#	O	O#	X	O#	O#
INTOTO MARYAM	O	O	Rare@	X	@	O#	O#	O	@	X	O#	O#

TABLE 22. SUMMARY OF DATA ON R. MOOSERI INFECTION (BY DIRECT FA TEST) IN ECTOPARASITES FROM VARIOUS AREAS  
 IN ETHIOPIA (1976-1977)

+ = Some samples found positive. (NOTE: A few L. segnis were found on Praomys and were negative.)  
 O = Samples tested were negative. @ = Species present, but not tested. # = Limited sampling.  
 X = Not collected and may be absent.



may be a concatenation of events responsible for the initiation and maintenance of a focus of this rickettsiosis, involving some or most of the above factors and some as yet unknown.

3) The multiple, concurrent R. mooseri infections in 2 or 3 species of fleas, at times accompanied by the presence of the rickettsiae in lice, and coupled with the presence of antibodies to murine typhus in the host Rattus strongly suggests that highly localized mini-foci of this infection exist, as in a hut or building. The fact that the great majority of Rattus in such loci harbor R. mooseri infections emphasizes the potential epidemiological importance of such minifoci, as does the fact that they persist for at least a year (the period of observation). If conditions in Ethiopia improve so that we could resume our investigations in that country, it would be edifying to learn if the Mekanissa barn and Amhara's house still are in the minifocus category, and to check sera of the people living or working there to determine the extent of human infection.

4) L. segnis merits extensive investigation as a vector or source of infection with R. mooseri, both with respect to man and murines. Its absence in Koka despite the presence there of a few infected Rattus may not be as significant as it appears. Thus, as mentioned above, those Rattus may have been introduced into the hotel and restaurant where they were trapped, since many trucks from Addis stop there. The very low rate of infection in Koka Rattus (3 of 106, = 3%, overall) suggest such a transient or tenuous endemicity, and a habitat where the infection cannot be readily maintained (and where presumably L. segnis cannot survive). This belief is supported by the failure to demonstrate R. mooseri in FA tests involving 110 X. cheopis and X. bantonum on Koka Rattus, and in 90 Echidnophaga from those rats in the few houses under study there, and by the dearth of Xenopsylla on rats in those domiciles. At Addis and Intoto, L. segnis outnumbered X. cheopis and X. bantonum by 6 to 30 times on Rattus, and generally had an R. mooseri infection-rate that was double that of the Xenopsylla. Even if this species does not bite man it may be a critical factor in the rickettsiosis as 1) an intramurid vector and 2) a source of infection in man and rodent by means of aerosol transmission of R. mooseri via its dried feces. L. segnis may be highly specific to Rattus and Mus, as indicated by the sparsity of records from other hosts (e.g. Praomys, Desmomys) in the same hut, but aerosol transmission of contaminated flea-feces could occur regardless.

5) The low interchange of fleas among the murines in the huts at Intoto Kedani Mehret is of great interest. This is the only area where we have found Rattus in the houses together with Desmomys, Praomys etc. In Uganda Hopkins (1949) noted that when Rattus first penetrate houses, it is restricted to the roofs, while Mastomys lives in burrows in the floor. This may also be the case in parts of Ethiopia, but, as we reported last year, at Menagesha, which is only 30 km. from Addis, there were no Rattus in the domiciles, only Praomys and Desmomys etc. This is apparently also true at Intoto Maryam just 4 km. from Addis, but at the lower village on Intoto, merely 2 km. from the capital, the Rattus and native murines are present in the same building. Here the Rattus do tend to occur in the roof and rafters, but a few were taken on the ground. There thus does not seem to be a physical barrier between the Rattus and Praomys etc., although the various kinds of rats may not frequently come into direct contact. It is noteworthy that the Leptopsylla aethiopica, Ctenophthalmus, Xiphiopsylla, Chiastopsylla, Dinopsyllus and other fleas of the indigenous murines in those domiciles were not found on the Rattus tenants, and that the L. segnis of the latter were rarely found on the Praomys, Desmomys, etc. Since L. segnis is supposed to be a Mus musculus flea, and yet can have an index of 50-90 on a Rattus, it is not a wholly specific flea, but its virtual absence on the native murines in the same building is noteworthy, particularly since the Xenopsylla freely share all of these murines. It may be that the dearth of L. segnis on Praomys and the other indigenous rats may have something to do with the low rate of R. mooseri infection in those rodents.

6) Roberts (1936) suggested that in Kenya X. brasiliensis is essentially a flea of rats nesting in the roofs, and X. cheopis, of rats living in the walls and floors. X. brasili-

liensis has not been found in Ethiopia and we do not know where X. cheopis (or X. bantonum) breeds indoors in that country. If some are developing on the ground-level (and we consider this possible because the eggs are not fixed to the hairs of the host and can fall to the ground), the newly emerged fleas would be in a position to readily infest Praomys and the other native murines. However, Rattus rattus in the Ethiopian areas under study definitely do tend to live in or near the roofs. Hopkins (1949) reported that 1) "where Rattus rattus is numerous other species of rats are not able to occupy permanent quarters in huts in any considerable numbers" and that this species "will invariably colonize thatch when it is available and is only infrequently found in the walls and floors of thatched huts." The second point certainly applies to the Rift Valley and parts of the Ethiopian highlands, but the huts at Intoto were made of galvanized metal or tin. The first point may also be applicable, and it seems likely that at Intoto Rattus has not become firmly established, having either recently entered, or more probably, being at its physiological threshold regarding existence at cold temperatures. The resulting degrees of contact between Rattus, and its flea-fauna, - and Praomys, Desmomys etc. and their fleas, - and the degree of specificity of these fleas, are no doubt important in the ecology of murine typhus.

7) The difference in the rate of R. mooseri infection in juvenile versus adult Rattus (Report p. 20, above) also is of epidemiological significance. It is to be expected that adult rats would have a higher rate of infection because 1) antibodies persist for life and 2) they have had more chance of coming into contact with the rickettsiae. That there is a difference, however, indicates that the sole source is not in the nest of the parents at time of birth or while the young are in the nest, or by acquisition of rickettsiae from the mother while in utero. The relatively high rate of infection in juveniles (44%) does indicate the theoretical possibility that both those last-mentioned factors may exist to a significant degree, and the subject warrants study. The fact that males and females become infected to the same degree (Report p. 18 and Table 10) shows that both sexes are probably equally exposed, but does not rule out such possible factors as stress of pregnancy, or the time spent in the nest by nursing mothers.

1976 and 1977

8) The differences in the rates of R. mooseri infection in both Rattus (Table 7, Report p. 16) and its fleas (Table 14, Report p. 23) are of interest. The fact that 63% of the town rats were positive in 1977 versus 49% in 1975-76 no doubt reflects our having concentrated more this year on the hyperendemic minifocus in Amhara's house than previously. Similarly, the 10% decrease in the rate at the barn at Makanissa, if significant, also may represent sampling. This year we trapped more heavily in an outbuilding there, where fewer positive rats were found. The differences concerning fleas are at a greater level and are more difficult to explain. Regarding town Rattus, in Xenopsylla the rate fell from 33% to 0 and to 3% in 1977, depending on the species; and Leptopsylla segnis, from 46% to 10%. A similar decrease was also noted at Makanissa. Since the majority of the fleas (and rats) came from minifoci that were hyperendemic a year previous, it is possible that the decreases represent a localized waning of endemicity. Another possible factor is that this year the rains lasted longer than usual and/or there were heavy rains immediately before we started operations. As mentioned above, such environmental conditions seriously affect the flea populations, which were lower than usual, and it may be that the fleas we had tested were unusually youthful (as the small numbers of mature eggs noted clearly indicate) and hence they had relatively little chance of acquiring R. mooseri infection, either due to having fed previously only on a few hosts, or because they had recently emerged, and had relatively few rickettsiae growing in their intestines, if indeed they had acquired any.

k. Publications on Murine Typhus Based on this Contract

1) Our extensive and critical review of the ecology of murine typhus, containing original concepts and ideas, and including well over 500 references, is being published by the Tropical Diseases Bulletin (Traub, Wisseman & Farhang-Azad, \*# in press). It will consist of

at least 70 pages. Invitational lectures on that subject were presented at the 15th International Congress of Entomology in Washington in 1976 and at the International Conference on Fleas at Ashton, England in 1977. The abstract from the Congress is already published (Traub, Wisseman & Farhang-Azad, \*#1977) and the other is in press (Traub, Wisseman & Farhang-Azad, \*# in press).

### 3. TICK-BORNE RICKETTSIOSIS - RICKETTSIAE OF THE SPOTTED FEVER GROUP

#### a. General Findings Regarding Infection in Rodents

1) Our previous reports showed that the spotted fever group of infections is widespread geographically among native rodents in Ethiopia. Data on the sera collected in 1977 are not yet ready for analysis, although below we mention evidence for presence of the rickettsiae in a pool of ticks. This rickettsiosis is considered so potentially important in Ethiopia that we review the observations to date.

2) The available data on IFA tests with rodent blood are summarized in Table 23, whence 3 important points become immediately apparent: 1) There is a very high rate of infection in "wild" rodents in the Koka area; 2) Rattus seems to play no role in this tick-borne rickettsiosis in the areas studied, and 3) there is no evidence to date of natural infection in either commensal or sylvan rodents in the Addis area.

3) A total of 22 of 92 (24%) Arvicanthis and 15% of 33 Mastomys at Koka had antibodies of the spotted fever group, whereas in the fields of the Addis suburbs (Area I.5 of Table 1) there were no infected Arvicanthis noted out of a total of 49 examined. This anomaly is difficult to understand and may be significant concerning understanding the ecology of this infection. As indicated below, a notable proportion of the ticks from these rodents, including species found positive for these rickettsiae by the FA test, feed on cattle during the adult stage of their life cycle. The acacia groves of Koka are daily visited by herds of cattle and goats, and this no doubt helps account for the relatively large tick population there on rodents. However, there are cattle and horses in the Addis suburbs as well, e.g. at the Mekanissa barn (I.3), but ticks were very scarce on rodents in the environs. Cattle ticks, however, abound. It might be thought that since the fields near Addis are under cultivation and are plowed, while acacia groves are unplowed, the difference in the tick population (and the incidence of naturally infected ticks and rodents) is due to the annual modifications of the environment. This may very well be a critical factor, but there are sections near Addis which are not disturbed by agriculture, and here the tick population has been low as well (although the sampling has been rather limited). Another possible reason may be that it is notably cooler at Addis than at Koka, and this possibility is strengthened by the observation that no rodent ticks were collected at Ankober (Area V), where it is definitely cold because of the high elevation. However, cattle were not examined at Ankober. Moreover, in Pakistan we had collected ticks and demonstrated tick-borne rickettsiosis in the high mountains, even in alpine terrain (#Robertson et al.; 1970; #Robertson & Wisseman, 1972). (Ticks from cattle from Addis and Koka were tested in limited number in 1977 and were negative, as noted below. Since it is prohibited to import cattle sera into the U.S., we have not been able to obtain data on the infection rate in cattle.)

4) The absence of tick-borne rickettsial infection in commensal Rattus (both Addis area and Koka town) is striking, and this is probably associated with the fact that none of the 190 such Addis Rattus examined had any ticks whatsoever, and that ticks were rare on the Koka town rats. A few ticks were collected on Rattus in the scattered huts in the fields of Koka in 1977.

5) Native murines (Praomys) were serologically found infected with those rickettsiae at Lemi and Meragesha as well, viz 1/28 (4%) and 1/19 (5%), indicating that tick-borne



HOSTS	ADDIS ABABA			KOKA-RIFT VALLEY		LEMI	MEMAGESHA	ANKOBER	TOTALS
	TOWN (HOUSES)	SUBURBS BUILDINGS	FIELD	TOWN	FIELD AREA				
RATTUS RATTUS	0/38	0/63	X	0/18 (7 in huts)	0/8	X	X	X	0/127
ARVICANTHIS SP.	X	X	0/49	X	22/92 = 24%	0/10	@	0/17	22/168 = 13%
DESMOMYS	X	X	X	X	X	0/5*	@	X	0/5
LOPHUROMYS	X	X	X	X	X	0/2*	X	0/8*	0/10
MASTOMYS NATALENSIS	(?)	@	@	@	5/33 = 15%	0/15	0/14	X	5/62 = 8%
MUS (LEGGADA)	X	X	0/4*	X	X	0/1*	@	@	0/5
OTOMYS TYPUS	X	X	X	X	X	X	X	0/8*	0/8
PRACOMYS SP.	X	X	X	X	X	1/28**=4%	1/19**=5%	X	2/47 = 4%
STENOCEPH- ALEMYS SP.	X	X	X	X	X	X	X	0/1*	0/1
TATERA ROBUSTA	X	X	@	X	0/2	X	X	X	0/2
TOTALS	0/38	0/63	0/53	0/18	27/135=20%	1/61=1.6%	1/53 = 3%	0/34	29/435

TABLE 23. RESULTS OF INDIRECT FLUORESCENT ANTIBODY TESTS FOR INFECTIONS OF THE SPOTTED FEVER GROUP, USING  
BLOOD COLLECTED ON FILTER PAPER, FROM SPECIFIED RODENTS IN PARTS OF ETHIOPIA (1975-1976)

Numerator = Number positive. Denominator = Number tested. X = Rodent not collected and may be absent.  
@ = Rodent present, but not tested. (?) = Not collected, but expected to be present. \* = Tested with  
fluorescein-conjugated antiserum to Arvicanthus globulin. \*\* = Ditto re. Mastomys.

rickettsiosis may be widely distributed in Ethiopia. The low rate of infection in those areas as compared to Koka is interesting but may be due to limitations in sampling.

b. Rickettsiae of the Spotted Fever Group Occurring in Native Commensal Rodents

1) Further analysis of the new data disclose a point fraught with epidemiological significance, namely that wild murines entering dwellings or acting as commensals, and their ticks, may be infected with these rickettsiae. The particulars on the murines are shown in Table 24. In all, 4 genera of wild rodents were found in huts and other domiciles in some of our study-sites,

HOSTS	KOKA FIELDS		LEMI		MENAGESHA		SUBTOTALS	
	HUTS	OUT-DOORS	TOWN BLDGS.	FIELDS	VILLAGE BLDGS.	FIELDS	BLDGS.	OUT-DOORS
ARVICANTHIS	8/26 =30%	7/8 =87%	Not Found	0/10	Not Found	Not Done	8/26 =30%	7/18 =40%
MASTOMYS	2/18 =11%	3/15 =20%	0/13	Not Done	0/16	Not Done	2/31 = 6%	3/15 =20%
NATALENSIS	Not Found	Not Found	1/24 = 4%	0/4	2/12 =17%	0/7	3/36 = 8%	0/11
PRAOMYS	Not Found	Not Found	0/5	Not Done	0/1	0/2	0/6	0/3
DESMOMYS	10/44 =23%	10/23 =43%	1/42 = 2%	0/14	2/28 = 7%	0/9	13/99 =13%	10/47 =20%
SUBTOTALS								

TABLE 24. NATURAL INFECTION WITH RICKETTSIAE OF THE SPOTTED FEVER GROUP OCCURRING IN "WILD" SPECIES OF NATIVE MURINES ENTERING OR LIVING IN HOUSES AS COMPARED WITH THOSE IN THE FIELD IN ETHIOPIA, 1976, AS SHOWN BY THE IFA TEST

Numerator = Number positive. Denominator = Number tested.

as mentioned above, and the dwellings at Koka in particular were so poorly constructed that Arvicanthus and Mastomys could readily enter, if they were not already living in the burrows commonly seen in the earthen floor of the huts. At Koka, 30% of the 26 Arvicanthus collected in huts were infected with these rickettsiae, as were 11% of 18 Mastomys. At Lemi, 37 Mastomys and Praomys were trapped inside of homes (which were quite well built), and 1 Praomys was serologically positive. At Menagesha 2 of 12 such Praomys (17%) were infected. Of the 4 genera of rodents found in buildings in these localities, all but Desmomys were thus incriminated with tick-borne rickettsiosis.

2) Table 25 also presents data on the murines trapped out-of-doors in those same areas, and at Koka the rate of natural infection was very high indeed among such Arvicanthus and Mastomys, viz. 7/8 (87%) and 3/15 (20%) respectively.

3) Available sera from some native murines at Lemi, Menagesha and Koka were tested in 1977 and the results are shown in Table 25. In all, 18% of the sera (21 of 114 samples) were

(TABLE 25 ABOUT HERE)

positive, with the rate ranging from 4% to 40%, while 15 of the 21 positives (71%) had titers of 1:80 or better.

c. Natural Infection with Tick-borne Rickettsiosis in Ticks and Mites

1) Last year we reported that 6 of 38 batches (16%) of ticks from various hosts and localities were naturally infected with this agent. The rate for individual ticks was 19/166 (11%). Of these, 2 of the 67 ticks (3%) tested from Koka were positive, but it is noteworthy

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HOST & LOCALITY	NO. AND % POSITIVE (THESE TESTS)	NUMBERS AND PERCENT AT VARIOUS TITERS					
		1:20	1:40	1:80	1:320	1:1280	1:2560
<u>PRAOMYS</u>							
LEMI	1/28 4%			1			
MENAGESHA	2/19 10%			1	1		
<u>MASTOMYS</u>							
KOKA	5/33 15%		2	1	1	1	
<u>ARVICANTHIS</u>							
KOKA	13/34 40%		4	5	3	1	
TOTALS	21/114 18%		6	8	5	2	

TABLE 25. POSITIVE TITERS FOR SOME SAMPLES OF RODENT SERA COLLECTED  
 IN ETHIOPIA IN 1976 AND TESTED IN 1977 BY IFA FOR THE  
 SPOTTED FEVER GROUP OF RICKETTSIAE

that both of these (1 Haemaphysalis larva and 1 Rhipicephalus larva) were from an Arvicantis collected in a hut. Thus, not only are there infected murines in the homes of the local people there, but ticks as well. Murines are of course associated with a variety of serious infections transmissible to man, and the abundance of native murines and Rattus, and their fleas, etc. in dwellings in Ethiopia is undoubtedly a matter of importance in public health.

2) Another dramatic finding in 1976 was that 13 of 13 (100%) Hyalomma larval ticks, collected from 2 thrushes, Myrmecocichla melaena (9 ticks and 4 ticks respectively) at Lemi were positive for rickettsiae of the spotted fever group. The role of birds in the ecology of this rickettsiosis is not well understood, but this observation indicates that birds may be important participants in Ethiopia (and if this thrush is migratory, in Asia as well - or wherever else it is the bird travels when migrating). The high rate of infection in the Hyalomma ticks suggested that this genus may be a major vector of the infection. (It is known that transstadial and transovarial transmission of Rickettsia sibirica may occur in Hyalomma asiaticum, fide Hoogstraal, 1967, and Hyalomma ticks have also been mentioned regarding R. conori ) Since the birds were collected 4 days apart, it may very well be that they had been infested at different times or places, even though the thrushes had been trapped within a mile of each other. All of the points are well worth further study.

3) The other positive results concerning our FA tests of ticks in 1976 concerned adult cattle ticks, i.e. Boophilus decoloratus (7% of 30) at Koka, Hyalomma marginatum rufipes (2 of 5 = 40%) at Lemi. A total of 15 Amblyomma and Rhipicephalus simus cattle ticks from Koka were negative. Philip, Hoogstraal et al. (1966) had reported isolations of R. conori from Amblyomma variegatum and Rhipicephalus simus from cattle in various parts of Ethiopia, including the Koka region, and also claimed serological evidence implicated other species of Rhipicephalus, H. v. rufipes and B. decoloratus. In 1975 we demonstrated rickettsiae of the spotted fever group in 2 of 2 Ixodes nymphal ticks from Suneus and 1 of 20 Rhipicephalus larvae, from Koka.

4) The available data on ticks, laelaptid mites and the spotted fever group of rickettsiae for 1977 are presented on Table 19 (Report p. 29), but specific identifications on these ectoparasites are pending. Out of 195 ticks from the areas and hosts listed, representing



17 batches, only one individual was positive, namely a tick from Herpestes (a mongoose) at Koka. As yet, no ticks nor rodents have been found positive for these rickettsiae in the Addis area, although endemicity was obviously widespread in Ethiopia, as indicated above. It therefore was particularly gratifying to find 4 of 10 laelaptid mites positive on Desmomys from Intoto, especially since the murine was trapped within a hut. In 1975 we reported positive mites from a shrew at Koka. Last year we also cited data on infected ticks and rodents within huts at Koka. It may be of importance in public health that people in various parts of Ethiopia are sharing their homes with ticks, mites and murines infected with rickettsiae of the spotted fever group.

#### 4. OTHER RICKETTSIAL INFECTION

##### a. Chigger-borne Rickettsiosis

1) There has been no definitive evidence that this infection occurs in Africa, and serological reports purporting to show endemicity cannot be properly evaluated (Traub & Wisseman, #1974). In our studies in Ethiopia we have collected 2 new species of the subgenus Leptotrombidium allied to the known vectors of this rickettsiosis (as well as several other new species of that genus and other genera). These constitute the first records of the vector-group in Africa, and since chigger-borne rickettsiosis has been found wherever members of this group of chiggers have been adequately tested, the subject is worth pursuing.

##### b. Epidemic Typhus

1) Severe cuts in the budget and the absence of a clinician in Addis made it necessary to suspend research on this program. However, with the arrival of Dr. Hamory on the scene at NAMRU-5, and his active interest and excellent work on this subject, investigations were renewed, but the departure of NAMRU-5 has forced termination of the clinical aspects of a highly promising study. Results are pending on the study of umbilical cord sera and maternal antibodies based upon material kindly collected by Dr. Sisay at Addis in collaboration with NAMRU-5.

2) The two articles by McDade et al. #, previously mentioned, have been revised and are in press. Other papers on epidemic typhus, not directly sponsored by this Contract are mentioned on Report p. 41 below.

#### 5. NOTES ON ZOOGEOGRAPHY

a. The observations on zoogeography this year are in accord with what we had reported last year. There are broad implications in the distribution of ectoparasite-borne infections and so we provide background material in this regard.

b. It was previously pointed out that in much of Ethiopian highlands the small mammals (theraphions) and their ectoparasites represented what we termed "faunal islands of discontinuity", i.e. they are relicts of ancestors which had a broad and continuous range in eras when conditions were quite different (cooler and moister) and the land had forest cover, and whose descendants have managed to survive even though the original habitat has been destroyed by man, livestock or increasing aridity. Ecological isolation has resulted in the formation of new species or subspecies, but the affinities with the montane fauna of Kenya, Tanzania and the Sudan are clear. It was also suggested that, as we have shown in Pakistan (Traub & Wisseman#, 1968, 1974; Traub#, 1972A and B), ectoparasite-borne or rodent-borne infections present in one mountainous area would be found in others, the intervening lowland desert or rain forest notwithstanding. Specific mention was made of tick-borne rickettsiosis, even though known African records pertained to warm semi-desert areas like Koka, and we predicted endemicity in the mountains because 1) we regard this as a very ancient infection (Traub & Wisseman#, 1974) and 2) we had found it widely distributed in a variety of habitats in Pakistan, including high mountains (Robertson et al. #, 1970). The data in 1976 supported these contentions, vide the demonstration of rickettsiae of the spotted fever group in ticks and rodents in Lemi and/or Menagesha, in the

highlands. We this year have reported endemicity on Mt. Intoto, just 2 km. from Addis, but in mites on a native murine in a house.

c) The fleas collected also provide similar evidence of an original continuous distribution followed by isolation in "islands" separated by ecological or topographical barriers. What is especially interesting is that the botanical environment has been so altered that today vast areas of terrain are uniform in appearance, and the former existence of varied habitats is suggested only by the affinities and features of the relict fauna. Thus, although there are no classical barriers like broad rivers, or lowland swamps or desert, or ranges of mountains between Ankober, Lemi, Menagesha, Addis and Mt. Intoto, there are conspicuous signs of former isolation, such as localized bioendemicity of fleas, mixed in with evidence of free and uninterrupted (and probably recent) passage between the areas. Some species of Ctenophthalmus like C. evidens range from East Africa through the Bale Mts. to Lemi, Ankober and Intoto on such wide-ranging hosts as Lophuromys and Dendromys, forming subspecies or sibling species en route in the passage of time. Others are quite distinctive and are apparently restricted to one of the study sites, although their affinities suggest similar roots and routes. Some Ctenophthalmus are utterly unlike their neighbors and show marked bioendemicity, and probably represent ancient stocks. Fleas of subterranean hosts like pocket gophers and mole-rats, which live in groups in restricted areas and show discontinuity in distribution, tend to show subspeciation and speciation in a marked degree. Their hosts often do too, but we believe the fleas proceed at a more rapid rate despite reports claiming that evolution in the host is faster than that of the flea (Dubinin, 1947). It therefore was to be expected that the mole-rat Tachyoryctes splendens has a different species of (Geoctenophthalmus) in Menagesha than at Ankober, as proved to be the case, but it was surprising to find 2 very different kinds of (Geoctenophthalmus) on this mole-rat in the same field at Ankober. The mole-rat flea at Intoto is the same species as at Menagesha, and other species are shared by these two mountains, which are only about 20 km. apart, but there may be significant differences at the subspecies level, indicating ecological isolation.

d) The finding of the Southern African genus of flea, Chiastopsylla, on the plateau of the Ethiopian highlands north of Addis and again at Intoto is unprecedented. There has been no previous indication of faunal connections between the two countries, save for high-ubiquitous hosts like Lophuromys, and if the Chiastopsylla had entered Ethiopia as a recent immigrant via such a mouse, then 1) there should be records of Chiastopsylla from East Africa, where fleas have been well studied because of plague - and there are no such reports - and 2) the 2 Chiastopsylla in Ethiopia should be known, widespread species, instead of being new species. These points suggest an ancient history in Ethiopia and lead us to believe that other such ectoparasites (or even mammals) of South African origin or affinity may be found, and that infections associated with those mammals, ticks and fleas, etc. in the southern part of the continent may exist in Ethiopia, e.g. a particular kind of tick-borne rickettsiosis. Viral infections in particular may be restricted to certain regions, e.g. Iassa Fever and Kyasanur Forest Disease, and while the reasons therefor are unknown, it is reasonable to believe they may be ecological or faunal, and hence may crop up in a "new" area where the prime requirements exist but not be recognized. It was this sort of reasoning that led us to search for, and find, the virus of Crimean Hemorrhagic Fever in W. Pakistan (Begum et al., 1970A, B).

#### 6. PUBLICATIONS BY THESE INVESTIGATORS

a. Allusion has been made above (section 2k, Report p. 34) to three papers, in press, dealing with the ecology of murine typhus and two (Report p. 39) on epidemic typhus.

b. The studies in Ethiopia have led to some valuable observations on the behavior of Leptopsylla segnis fleas, a species we believe may prove to be important in the ecology of

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murine typhus. These are reported in a paper on adaptations in fleas (Traub\*, in press).

c. There have been some recent publications prepared by the Responsible Investigator and members of this Department that are directly pertinent to the work being done under this Contract but which represent sponsorship by non-Navy sources. They are mentioned for documentation, for purposes of completeness regarding current state of the art, and so that the bibliography cited in this report is up-to-date and suitable for reference. As in the case of other relevant work done under other auspices by our staff, the items are marked with a #, and are as follows. The description of the plague assay technique for R. mooseri in tissue samples, which has proven so valuable in our Ethiopian studies, has just been published (Murphy, Wisseman & Snyder#, 1976). Beaman and Wisseman have been studying mechanisms of immunity in typhus infections, and an article dealing with R. mooseri-specific antibodies and one on the differential opsonizing and neutralizing action of human typhus rickettsia-specific cytophilic antibodies have just appeared (#1976a and b). A paper on the prevention and control of rickettsial diseases was presented at a meeting in Czechoslovakia in 1976 (Wisseman#, in press). Recent papers on epidemic typhus were prepared by Fabrikant, Wisseman et al.# (1973); and by Stork and Wisseman# (1976).

#### 7. SUMMARY OF PROGRESS REPORT

a. Considerable progress was made during the current year despite the facts that the bulk of the studies were based upon collections of specimens, observations and data made during just one month's field work in Ethiopia and that the Ethiopian Government restricted our activities to "Addis Ababa" and Koka. The new approach and methodology proposed for and utilized in this project have proven sound, and the serological results fully reliable when checked by standard tests.

##### b. The Ecology of Murine Typhus

1) The evidence continues to overwhelmingly suggest that in Ethiopia, murine typhus infection is intimately associated with introduced commensal rodents (Rattus rattus and Mus musculus), and their ectoparasites, indoors. Other commensal rodents like Mastomys, Praomys, which are native murines, do not seem to play a role in the absence of Rattus or M. musculus even though they may be common in domiciles and infested with Xenopsylla cheopis, the presumed vector, or with X. bantorum, a species which has also been found naturally infected with R. mooseri in our study. If Rattus or M. musculus co-exist with Praomys, then it (and probably other native murines as well) may become secondarily involved on a limited scale.

2) So far as known, murine typhus infection in Ethiopia is endemic only in areas where Rattus rattus and M. musculus occur, and if so, this means that the infection is greatly restricted both geographically and ecologically since these particular commensal murines have been found essentially only indoors and then in towns or along the main routes of commerce. This restriction implies that Rattus and/or M. musculus are either recent immigrants or are severely limited because of ecological reasons, i.e., extreme cold in the highlands (e.g. their absence at 2900 m. in huts on Mt. Intoto, only 4 km. from Addis, although present 2 km. away, at 2600 m.), or aridity in the outdoors at Koka, 100 km. SW of Addis, in the Rift Valley at 1640 m. elevation. Not only are some villages a few kilometers from Addis apparently free of Rattus (e.g. Menagesha) and demonstrable R. mooseri infection in the commensal murines (Praomys, Desmomyss, Mastomys, etc.) and their fleas (including X. cheopis and X. bantorum), but endemicity may vary considerably within the foci where R. mooseri occurs in Rattus and its ectoparasites. In Addis town and in its suburb Mekanissa, all the infected fleas and lice noted came from a "minifocus" in each area - Amhara's house or the barn. Moreover, the Rattus in the minifocus had an appreciably higher rate of infection than in other places in the same vicinity, viz, 78% in Amhara's house versus 58% elsewhere in Addis.

3) Non-commensal rodents were free of demonstrable R. mooseri infection, even in



the case of native murines living in fields adjacent to areas where infected Rattus were found in buildings, viz, at Mekanissa, where the rate was 0/49 in Arvicanthis but 41/51 (80%) in Rattus in the barn. In Koka town, 3/105 (3%) of the Rattus were positive by IFA, but none of the 99 Arvicanthis in the Acacia groves 5 km. south were infected, even though they were infested with 20-30 times as many X. cheopis fleas. Excluding Praomys, a total of 343 native rodents were tested and all of these were negative. 184 Arvicanthis and 83 Mastomys were included. There were 294 rodents from the outdoors which were examined by IFA tests, and all were negative, whereas 138 of 576 (24%) indoor rodents were positive for R. mooseri. Where Rattus and/or Mus were present indoors, the totals for all indoor murids, including Praomys etc. are 138/522, or 26%.

4) Xenopsylla cheopis was present wherever infected Rattus were found but was uncommon in the Addis area. Here the "index" or average number per rat was only in the vicinity of 0.3 at the period of maximal abundance (the dry season). In contrast in Koka, where it is much warmer, in 1976 the index on town Rattus was 6 times as high, but on Arvicanthis in the fields it was 150 times as abundant as at Addis. (We regard Arvicanthis as the true host and Northern Africa as the original home of this so-called Indian Rat Flea.) In all areas and on all hosts, X. cheopis was accompanied by a somewhat more abundant, sibling species, X. bantorum, whose native host we believe is Mastomys. In Addis Rattus was commonly infested with Leptopsylla segnis fleas, with an index of about 5 or 6. L. segnis is virtually restricted to Rattus or Mus musculus and has not been found in the other areas except for Mt. Intoto. A related species, L. aethiopica occurs on native murines (especially Mus (Leggada)) in all the localities studied, and was common on those hosts in houses in Lemi and Menagesha, where Rattus has not been found, but where X. cheopis and X. bantorum infest these Mastomys, Praomys or Desmoms, all in the absence of demonstrable R. mooseri.

5) Xenopsylla fleas exhibit a marked seasonal incidence in Ethiopia, viz, at Koka they are 100-fold as prevalent in the dry season as at the end of the rains. Since they are so uncommon at all seasons in Addis, although varying in an analogous way with respect to the rains, and since Xenopsylla were not collected at all at Ankober (at a much higher elevation), we believe that X. cheopis and X. bantorum are near their ecological limits in the climate of a highland city like Addis (approx. 2400 m.). This belief is supported by its rarity at 2900 m. on Mt. Intoto, especially outdoors.

6) Hyperendemic, highly localized foci have been noted in Addis. Thus, 87% of the instances where an infected louse or flea was detected by the FA test came from either Amhara's house or the barn at Mekanissa. Infected fleas and/or lice were found on 30 occasions, and in 12 instances more than one species of ectoparasite was tested from those rats. In 50% of those instances, more than one species on the rat was found to harbor R. mooseri. Further, an unusually large percentage of the individuals in the pool was positive. In several cases, 100% of the fleas or lice tested had rickettsiae. Rattus from these particular hyperendemic "minifoci" had a significantly higher incidence of infection than did those from other adjacent areas. It is emphasized that there was a high rate of infection in the barn in 1975-76-77 and in Amhara's house both of the years tests were made. Such persistence of infection in a focus needs special study.

7) The current findings emphasize the need for re-evaluation of the role of rat-lice (Polyplax and Hoplopleura) in the ecology of murine typhus. There are 2 species of lice on Rattus in Ethiopia, P. spinulosa and H. oenonydis, and both may occur on the same rat. Lice have been difficult to collect and in 1976 only 18 (from 13 Rattus) were tested by the FA technique, and 6 of these (33%) were positive. Both species were represented and some of each species were infected. Two of the lice (from 2 different Rattus) were from the same housing-compound where all the infected fleas had been collected. In 1977, 1 of 200 Rattus lice was positive, and it was from the same minifocus.

8) There have been no positives noted in direct FA tests for R. mooseri in a total of 273 ticks (195 this year) nor in 210 mesostigmatid mites (140 in 1977). Only 10 of the ticks were from Rattus, and only 60 of the mites came from that host.

c. Tick-borne Rickettsiosis

1) In the 1976 studies, 15 of 34 (44%) of the Arvicantis and 5 of 33 (15%) of the Mastomys in the Koka area were infected with rickettsiae of the spotted fever group. In marked contrast, none of the 49 Arvicantis from the Addis suburbs, examined during 1975-76 were positive. The reasons for such a discrepancy are unclear, particularly in view of our finding infected rodents (1/28 Praomys at Lemi and 1/19 Praomys at Menagesha) at highland elevations and terrain comparable to Addis. Data on rodent sera for 1977 are pending. The same general picture is presented by the data on ticks: In 1976, 3% of 67 ticks at Koka; 26% of 66 at Lemi, but 0/33 at Addis were positive. In 1977, only one tick of 195 tested was infected, and it came from Koka.

2) Notably some of these 1976 native murines were trapped in dwellings in Koka, Menagesha and Lemi, where they often are abundant. Thus, 30% of the 26 Arvicantis taken in huts at Koka were positive for these rickettsiae. The 2 ticks found infected at Koka in 1976 were also from 2 such commensal Arvicantis. This year's infected tick was from Herpestes at Koka.

3) Two thrushes at Lemi carried Hyalomma larval ticks of which all the samples tested (13/13) were positive for these rickettsiae. This suggests that both migratory birds and Hyalomma ticks may be major participants in the cycles of tick-borne rickettsiosis in Ethiopia.

4) In 1977, 4 of 10 laelaptid mites from a Desmomyss in a hut at Intoto were infected. In 1975 some infected mites were found on a shrew at Koka.

5) The occurrence in domiciles of rodents and ticks infected with these rickettsiae in various parts of Ethiopia is significant.

d. Other Points

1) Among the new species of chiggers collected by us in Ethiopia are two members of the subgenus Leptotrombidium related to the vectors of chigger-borne rickettsiosis in the mountains of Pakistan and northern Asia. Since infection with R. tsutsugamushi, the agent of that rickettsiosis, has been found wherever sought in the presence of that group of chiggers, and ours are the first records of such trombiculids in Africa, the possibility of chigger-borne rickettsiosis occurring on that continent should be investigated.

2) Because of cuts in the budget and the absence of a clinician in Addis, research on epidemic typhus was deemphasized. Work is continuing on serological studies on maternal and fetal antibodies.

3) Despite the destruction of the original vegetative cover (forest in the highlands) over much of Ethiopia, the Siphonapteran fauna is a fairly rich one, representing relict survivors of former conditions. Most of the highland species on native murines belong to Ctenophthalmus, and there are at least 10 species new to Science at hand. Many of the other non-commensal species are also new. In that category, and of particular interest are the Echidnophaga of house rats (Rattus) in Koka which burrows into the superficial layers of the skin of the host, and 2 Chiastopsylla from Lemi, Menagesha and/or Intoto. The latter genus has never been reported north of Rhodesia, and the family's northeastern record has been the Congo. Both of these new Chiastopsylla have been found on native murines within houses as well as in the fields.

4) The zoogeographic findings continue to be of significance. More data have been accumulated indicating the relict nature of the fleas in many of the highland areas, not only in the isolated mountains but in the plateaus where destruction of the forest cover has obliterated ecological barriers that defined microhabitats. Areas of the same general appearance

and of uniform, confluent, topographic features show surprising elements of bioendemicity. Much of the highland fauna is of East African montane affinity, but we have learned that fleas of South African origin are also present (i.e. 2 new species of Chiastopsylla at Leml) although such possible faunal relationships had not been suspected. One of these Chiastopsylla is common on native murines in dwellings and hence may prove to be of medical significance. The zoogeographic data on fleas led us to predict that infections associated with rodents and ectoparasites in one area probably occur in other areas where a similar fauna exists, despite "barriers" like semi-desert in between. Tick-borne rickettsiosis has now proven to be a case in point, and the finding, reported in 1976, of R. mooseri infection in Rattus in tropical lowland areas like Gambela is also in accord with these concepts.

5) The technique of isolating R. mooseri from the kidneys of a field-caught rat directly into tissue cultures has proven very successful.

6) A total of 6 publications, based wholly or in part upon this Contract or its predecessor, are in press.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The evidence continues to suggest overwhelmingly that, in Ethiopia, murine typhus infection is intimately associated with introduced comensal rodents ( <u>Rattus rattus</u> and <u>Mus musculus</u> ), and their ectoparasites, indoors. Other comensal rodents like <u>Mastomys</u> , <u>Praomys</u> , which are native murines, do not seem to play a role in the absence of <u>Rattus</u> or <u>M. musculus</u> even though they may be common in domiciles and infested with <u>Xenopsylla cheopis</u> , the presumed vector, or with <u>X. bantorum</u> , a species which has also been found naturally infected with <u>R. mooseri</u> in our study. If <u>Rattus</u> or <u>M. musculus</u> co-exist (OVER)		

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with Praomys, then it (and probably other native murines as well) may become secondarily involved on a limited scale.